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Association between oral lichenoid reactions and amalgam restorations

S Pezelj-Ribarić,*† J Prpić,† I Miletić,‡ G Brumini,§ M Šimunović Šoškić,¶ I Anić‡

† Medical Faculty, University of Rijeka, Department of Oral pathology, Rijeka, Croatia
‡ School of Dentistry, University of Zagreb, Department of Endodontics and Restorative Dentistry, Zagreb, Croatia
§ Medical Faculty, University of Rijeka, Department of Informatics, Rijeka, Croatia
¶ Medical Faculty, University of Rijeka, Department of Prosthetic Dentistry, Rijeka, Croatia

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*Corresponding author, Department of Oral pathology, Medical Faculty, University of Rijeka, Brace Branchetta 20, 51 000 Rijeka, Croatia, tel. +385 51 345 645; fax +385 51 345 630; E-mail: sonja.pezelj-ribaric@medri.hr

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Abstract

Background The aim of this study was to perform a clinical assessment of the association between oral lichenoid reactions (OLR) 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.

Methods The study included 20 patients with OLR and 20 healthy volunteers, who were examined between 2001 and 2005 at the Oral Medicine Unit of the Medical Faculty University of Rijeka. All patients were skin patch tested by an experienced physician. Saliva samples were collected, prepared and analysed for IL-6 and IL-8 concentrations using enzyme-linked immunosorbent assay.

Results Sixteen out of 20 patch-tested patients showed a sensitization to inorganic mercury or amalgam. Total replacement of all amalgam fillings was carried out on 20 patients with fillings based on composite resin, gold, porcelain or a combination of these. Sixteen out of 20 patients showed complete healing of OLR; three patients had marked improvement, whereas one patient showed no improvement. Levels of IL-6 detected before replacement were significantly higher than IL-6 levels following the replacement ($P = 0.003$). The IL-8 levels measured before replacement procedure were significantly higher than the IL-8 levels after replacement of the fillings ($P < 0.001$).

Conclusions On the basis of clinical observations, restorative therapy resulted in tissue healing. Following the replacement of amalgam fillings with fillings based on other restorative materials, levels of both IL-6 and IL-8 shifted towards normal, as measured in healthy subjects.

Introduction

Oral lichen planus (OLP) and oral lichenoid reactions (OLR) are chronic inflammatory lesions of the oral mucosa.[1] OLP has clinical and histological similarities to OLR but is a potentially premalignant disease affecting approximately 2% of the population.[2] Some authors consider a contact allergy to amalgam or other factors mentioned above to cause OLP, whereas others claim the existence of two separate diseases: OLR related to amalgam and OLP as an idiopathic disorder.[3] Clinically, OLP is in most cases bilateral and OLR unilateral. OLP is most often present on the buccal mucosa, gingiva and tongue. It can be presented in a number of forms including reticular, erosive, atrophic, plaque type, papular or bullous. OLR express more diffuse inflammation extending more deeply. Presence of plasma cells and occasional eosinophils are claimed to be diagnostic criteria in OLR.[1] Both lesions show liquefaction degeneration of the basal cell layer and hyperkeratinization of the epithelium. Drugs such as beta blocekrs, dapsone, oral hypoglicemics, non-steroidal anti-inflammatory drugs, penicillamine, phenothiazines, sulphonyureas and gold salts have been associated with lichenoid reactions.[4]

Associations between OLR and exposure to various dental materials, particularly dental metals, have been investigated by assessing allergic responses in patients using cutaneous patch testing.[5] Most researchers have found an increased incidence of allergic response to commonly used dental materials in patients with OLP over patients without mucosal disease. Other investigators have attempted to explore the contribution of dental materials to oral lichenoid lesions by removing dental restorations and monitoring the course of the lesion progress following removal.[6] Despite the benefits of amalgam fillings, there are growing concerns regarding the potential adverse health effects arising from exposure to mercury released from set amalgam. Mercury has been shown to accumulate in the oral mucosa, and, in some individuals, this can cause a chronic lichenoid reaction of the oral mucosa juxtaposed to an amalgam filling. Patients with amalgam-induced OLR are often hypersensitive to mercury compounds. The accumulation of a band-like infiltrate of leucocytes in the *lamina propria* adjacent to the basal keratinocyte layer with destruction of the basement membrane and basal keratinocytes is a feature of amalgam-induced OLR and OLP.[7]

Pezelj-Ribarić *et al*.[8] found significantly higher amounts of tumour necrosis factor-alpha (TNF-α) in all saliva samples obtained from patients with active OLP lesions in comparison with healthy controls. Moreover, these data showed that salivary concentrations of TNF-α varied in different clinical types of OLP, being particularly elevated in the cases of erosive/atrophic form of the disease. It is obvious that enhanced TNF-α production in saliva reflects clinical changes and correlates with the severity of OLP. Aim of this study was to:

1 examine the effectiveness of amalgam replacement, which is considered to be the cause of OLR and
2 evaluate salivary concentrations of interleukin-6 (IL-6) and IL-8 before and after replacement of amalgam restorations.

Materials and methods

Study involved 20 patients with OLR and 20 healthy volunteers, who were examined between 2001 and 2005 at the Oral Medicine Unit of the Medical Faculty University of Rijeka. We excluded patients taking drugs that might cause a lichenoid reaction and those with lesions on the skin or locations other than oral mucosa. All subjects were informed of the aims and procedures of the research, as well as of the fact that their medical data would be later used in the analysis. Within the research, they were guaranteed respect of their basic ethical and bioethical principles – personal integrity (independence, righteousness, well-being and safety) as regulated by Nûrnberg codex and the most recent version of Helsinki declaration. Only those subjects who have given a written permission in form of an informed consent were included.

Each subject completed a questionnaire for demographic and health information. The patients were diagnosed clinically and confirmed as having OLR by taking a biopsy, according to the criteria of the WHO.

Lesions

Two examiners performed all examinations and restoration analysis. OLR lesions of all patients were located adjacent to amalgam fillings. The most common site of involvement was buccal mucosa (14 cases), followed by the tongue (4 cases) and gingiva (2 cases). Clinical examination was performed according to the standard clinical criteria. Lesions were described as reticular if mainly lace-like hyperkeratotic patterns were present (12 cases), and erosive/atrophic if they exhibited erythematous change with a few aggressive ulcers (8 cases).

Histopathological examination

Specimens of OLR tissues obtained by taking a biopsy were stained with haematoxylin and eosin (HE staining) in order to evaluate the characteristic histopathological features of OLP. In each case, the clinical diagnosis was confirmed when a histological appearance included a band-like, mainly lymphocytic infiltrate in the connective tissue adjacent to the epithelial basement membrane, liquefaction degeneration of the basement membrane and destruction of the basal keratinocyte layer.[9]

Patch testing

All patients were skin patched tested by a physician experienced in skin patch testing. The following tests were performed: amalgam 5%, mercury ammonium chloride 1%, mercury 0.5%, thimerosal 0.1%, phenylmercuric borate 0.05%, phenylmercuric acetate 0.05%, and phenyllmercuric nitrate 0.05% incorporated in petrolatum (Hermal, Reinbek, Germany). Readings were performed after 1, 2 and 3 days in accordance with the recommendations of the International Contact Dermatitis Research Group.[10]

Saliva collection and cytokine assay

After informed consent had been obtained and medical, dental and social histories taken, the whole unstimulated saliva was collected between 9:00 and 11:00 a.m. using standard techniques described by Navazesh.[11] Participants refrained from eating, drinking, using chewing gum, etc. for at least 1.5 h prior to evaluation. Saliva specimens

were collected from each participant in the sitting position. Samples were obtained in the following manner: first, the subjects were requested to swallow, tilt their head forward and expectorate all saliva produced during 5 min into 50 mL tubes without swallowing. The final volume and flow rate of saliva were determined gravimetrically (Analytical Balance, Model WTS-6001, Sartorius Corp., Long Island, NY).[12] The entire procedure was repeated 3 months following replacement of the amalgam fillings with composite/other materials. Saliva specimens were stored at –80 °C until the beginning of analysis. For determination of salivary levels of IL-6 and IL-8, enzyme-linked immunosorbent assay (ELISA; Sigma Immunochemicals, St Louis MO) was performed according to the manufacturer's instructions, and the results were expressed in pg/mL. The test was performed in duplicate and repeated three times. Protein content was expressed in pg/mL.

Amalgam replacement

Twenty patients in this investigation had their amalgam fillings replaced with composite resin, gold, porcelain or a combination of these. After a follow-up period of between 2 months and 3.5 years, these patients were evaluated in the clinic. OLR extent and severity was graded as:

1 healed (no lesions remaining)
2 marked improvement (> 80% improvement)
3 no improvement or worsening of the symptoms (increasing OLR)[6]

There were a total of 16 women and 4 men. There were no significant differences in age between the diseased and control group (50 ± 9 vs. 51 ± 9 years; $P = 0.716$).

Statistical analysis

Patients' ages in both groups are presented as mean value^{+}-standard deviation. Differences in patients' ages were analysed using one-way ANOVA. Data on IL-6 and IL-8 are presented as median values and interquartile range (IQR). The results were compared using non-parametric Wilcoxon test for dependent samples and Mann–Whitney *U*-test for independent samples. Analysis of the presence of various histopathological features was performed using Pearson's χ^2 test.

Differences were considered significant at the *P* level of < 0.005. Statistical analysis of data was performed using Statistica for Windows, release 6.1 (StatSoft, Inc., Tulsa, OK).

Results

Sixteen out of 20 patched-tested patients showed a sensitization to inorganic mercury or amalgam. Four patients showed a positive reaction to at least one of these organic derivates of mercury.

Table 1 Stratification of lesion improvement following removal of amalgam restorations

Grade	Frequency	Statistic
Healed (no lesions remaining)	16	$\chi^2 = 16.94$ $P < 0.001$
Marked improvement (> 80% improvement)	3	
No improvement or worsening of the symptoms	1	

Table 2 Detected values of IL-6 and IL-8 before and after filling replacement

Expression	Before		After		Control	
(10^{-2} pg/mL)	Median	IQR	Median	IQR	Median	IQR
IL-6	43.1	24.1–55.4	0.5	0.2–2.2	0.3	0.1–1
IL-8	58.1	43.5–77.6	0.3	0.9–12	0.7	0.4–8.6

Complete replacement of all amalgam fillings was carried out on 20 patients with fillings based on composite resin, gold, porcelain or a combination of these. Sixteen out of 20 patients (80%) showed a complete healing of OLR; three patients presented a marked improvement, whereas one patient had no improvement whatsoever. One hundred per cent regression of the changes was significantly the major outcome, compared with other categories (Table 1). Patients with a positive patch test reaction to amalgam showed complete healing. Saliva samples were taken from all patients before and after replacement of amalgam fillings. Levels of the pro-inflammatory cytokines IL-6 and IL-8 in whole saliva samples were determined using ELISA. Levels of IL-6 measured before replacement of the fillings were significantly greater than IL-6 levels following replacement ($P = 0.003$). Levels of IL-8 detected before replacement were also significantly greater than the subsequent IL-8 levels ($P < 0.001$).

Levels of IL-6 measured before and after intervention in control subjects did not differ significantly ($P = 0.226$). The same applies to IL-8-values ($P = 0199$). Results of filling replacement show that the values of pro-inflammatory cytokines returned to levels that correspond to those in control group. Restorative therapy lowered the values towards normal (Table 2, fig. 1).

Discussion

Lichenoid reactions as an allergic reaction to dental materials have been widely reported. Many studies have documented contact hypersensitivity to dental materials

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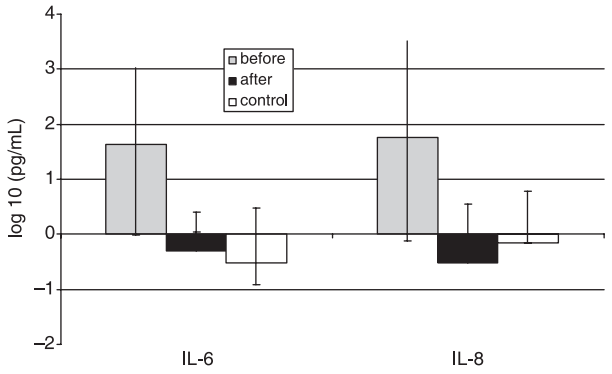

fig. 1 Graphic representation of detected values of IL-6 and IL-8 before and after filling replacement.

such as amalgam, composite and dental acrylics presenting as lichenoid reactions. In most cases, OLR are indistinguishable from idiopathic OLP, clinically or histologically.[13] Previous studies have shown that subjects with OLP showed significantly higher expression of nuclear factor-κB (NF-κB)-dependent cytokines in serum, oral keratinocytes and tissue-infiltrated mononuclear cells, including TNF-α, IL-1 and IL-6.[12] Amalgam has been used as a dental restorative material since 1831. Its positive characteristics include strength, longevity, good marginal adaptation, easy handling and price. The pathogenic relationship between OLR and dental amalgam fillings is still a matter of controversy. Several studies suggested that such restorations may induce a lichenoid reaction in oral mucosal tissues in susceptible patients,[14] and that a high percentage of the lesions improved following removal of amalgam fillings, although not all patients showed the same response.[15] In their study, Little *et al*.[7] found no expression of IL-8 by oral mucosal cells in either amalgam-induced OLR or OLP. They used standard immunoperoxidase techniques to visualize the expression of this molecule in frozen biopsy sections. Present research was performed on the whole saliva samples using EIA; therefore, the difference in the obtained results can be explained by different methodology.[16] Notwithstanding, Sun *et al*. proved in their research that serum IL-8 levels can be an objective marker in monitoring various stages of OLP.[17] In addition, the study by Maie *et al*.[18] showed that salivary IL-8 can serve as a biomarker in confirming oropharyngeal squamous cell carcinoma. In this study, with a small sample size, we found a statistically significant reduction in salivary levels of pro-inflammatory cytokines IL-6 and IL-8 in patients who underwent amalgam filling replacement with fillings based on composite resin, gold, porcelain or a combination of these. These results were supported by clinical changes. In particular, during the course of our study, a marked improvement was observed on the oral mucosa, where 16 patients presented a complete healing, and 3 cases (15%) presented a significant improvement. In one patient, no changes on the oral mucosa were observed following replacement of the fillings.

This study confirms that OLR can be caused by, or associated with an allergy to mercury in amalgam fillings. These results concur with previous studies reporting mercury allergy in 16% to 64% of OLR patients.[16] Both IL-6 and IL-8 were successfully detected at significantly elevated levels in three types of oral fluids from OLP patients, and the change in NF-κB-dependent cytokines in saliva partially reflected the trend of malignant transformation of OLP in study performed by Rhodus *et al*.[12] Their results showed that concentrations of all the mentioned cytokines were elevated in patients with OLP in all examined oral fluids, which is consistent with our findings. Similar research was performed by Rhodus *et al.* with the exception that samples were taken from tissue transudate; nevertheless, the results showed increased concentrations of TNF-α, IL-1α, IL-6 and IL-8.[19] It can be noted that, using different oral fluids, various authors proved IL-6 and IL-8 to be potential biomarkers in OLP monitoring. In our investigation, restorative therapy yielded tissue healing as assessed by clinical parameters. Salivary IL-6 and IL-8 were also important indicators, showing a statistically significant difference before and after filling replacement. Replacement of amalgam fillings with fillings based on other materials resulted in a shift of cytokine levels towards normal values (as measured in healthy subjects).

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