Toxicology Letters 211 (2012) 120–125

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Minor changes in serum levels of cytokines after removal of amalgam restorations☆

Lars Björkman[a,b,*], Karl A. Brokstad[c], Ketil Moen[b,d], Roland Jonsson[c]

[a] Dental Biomaterials Adverse Reaction Unit, Uni Health, Uni Research, Årstadveien 17, NO-5009 Bergen, Norway
[b] Department of Clinical Dentistry, University of Bergen, Årstadveien 17, NO-5009 Bergen, Norway
[c] Broegelmann Research Laboratory, the Gade Institute, University of Bergen, Laboratory building, 5th floor, Haukeland University Hospital, NO-5021 Bergen, Norway
[d] Department of Oral and Maxillofacial Surgery, Haukeland University Hospital, Jonas Lies vei 65, 5021 Bergen, Norway

ARTICLE INFO

Article history:
Received 27 January 2012
Received in revised form 18 March 2012
Accepted 20 March 2012
Available online 28 March 2012

Keywords:
Cytokine
Serum
Dental amalgam
Human
Clinical trial

ABSTRACT

Dental amalgam restorations release mercury and silver which is absorbed and distributed in the body. Animal studies have shown that both elements may interfere with the host by activation of the immune system in genetically susceptible strains at exposure levels relevant to those from dental amalgam restorations. The aim of this study was to test the hypothesis of no change over time in concentrations of a number of immune mediators in serum after removal of all dental amalgam restorations in patients with health complaints attributed to their amalgam restorations and compare with a healthy reference group. Twenty patients previously examined at a specialty unit for health complaints attributed to dental materials were included in a clinical trial and had all amalgam restorations replaced with other dental restorative materials. Serum samples were collected before amalgam removal and 3 and 12 months after the removal was finished. Twenty blood donors matched for age and gender were used as comparison group. A fluorescent bead-based (Luminex) immunoassay kit was used to measure cytokines, chemokines and growth factors in serum. At baseline, the patient group had slightly higher values for GM-CSF, IL-6, IL-2R, IFN-alpha, IL-7, and IL-12p40/p70 compared with the reference group. After amalgam removal a decrease towards the median value of the reference group was found for GM-CSF, IL-8, and IL-7. In conclusion, removal of all dental amalgam restorations and replacement with other dental restorative materials was associated with decreased concentrations of Th1-type proinflammatory markers in serum.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Dental amalgam restorations release low levels of mercury and silver, which is absorbed and distributed in the body (Berglund, 1990; Björkman and Lind, 1992; Björkman et al., 2007; Drasch et al., 1995; Gay et al., 1979; Lygre et al., 1999; Nylander et al., 1987; Richardson et al., 2011; Skare and Engqvist, 1994; Vimy and Lorscheider, 1985). Studies on rodents have shown that both elements may interfere with the host by activation of the immune system in genetically susceptible strains at exposure levels relevant to those from dental amalgam restorations (Hultman et al., 1994, 1998; Pollard et al., 2001). In addition, in vitro exposure of human peripheral blood mononuclear cells to low concentrations of mercury has shown effects on the release of a number of cytokines (Gardner et al., 2009; Hemdan et al., 2007).

In humans, it is well documented that corrosion products released from dental amalgam restorations may cause intraoral contact lesions (oral lichenoid lesions) in sensitive individuals. Removal of amalgam restorations in contact with the lesion is usually followed by improvement or complete healing (Issa et al., 2004). A study on patients with oral lichenoid lesions in contact with amalgam restorations showed that the concentration of both interleukin (IL)-6 and IL-8 (CXCL8) in saliva was reduced after removal of all amalgam restorations (Pezelj-Ribaric et al., 2008). Thus, there is support for a local release of immune mediators in individuals sensitive to amalgam restorations.

Some patients with amalgam restorations attribute health complaints to their amalgam restorations. Common health complaints in this group of patients are pain in joints and muscles, fatigue, memory problems, and intraoral pain and discomfort (Langworth et al., 2002; Lygre et al., 2005). Local intraoral contact lesions are not common in this group. Experimental studies on patients with health complaints attributed to amalgam restorations have shown

☆ The study was a part of a project approved by the Regional Committee for Medical Research Ethics in Western Norway (REK III, 24.01) registered at ClinicalTrials.gov (NCT00346944).

* Corresponding author. Tel.: +47 55586495; fax: +47 55589862.
E-mail address: Lars.Bjorkman@uni.no (L. Björkman).

0378-4274/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2012.03.769

a decrease of health complaints after removal of the restorations (Melchart et al., 2008; Nerdrum et al., 2004; Sjursen et al., 2011). However, the cause of the improvement is not known, but may be related to a number of factors associated with removal of the restorations (Sjursen et al., 2011). Since mercury concentrations in blood and urine in this group of patients are within normal limits (Langworth et al., 2002; Vamnes et al., 2004), mercury intoxication is not probable, but it has been hypothesized that some individuals may be more sensitive to mercury exposure (Anneroth et al., 1992; Strömberg et al., 1999), which could be of relevance for low level exposure from dental amalgam restorations.

The aim of the present study was to characterize serum cytokine levels before and after removal of all dental amalgam restorations in patients with health complaints attributed to their amalgam restorations using an open approach. In addition, concentrations of immune mediators in serum were compared with a healthy reference group.

2. Materials and methods

2.1. Study groups

Twenty patients previously examined by dentists and physicians at the Dental Biomaterials Adverse Reaction Unit in Bergen, Norway (Vamnes et al., 2004) were included in the study (Sjursen et al., 2011). The patients were referred from dentists and physicians for examination of health complaints attributed to amalgam restorations. At the examination, no causal relationship between amalgam exposure and health complaints was established, and thus, the patients were not recommended removal of amalgam restorations. No patients had signs of lichenoid contact lesions to amalgam restorations or other dental materials.

After information and written consent, the patients (6 men and 14 women; mean age at pre-treatment examination 49 years; SD 6.7) had all their amalgam restorations removed and replacement with other dental restorative materials under controlled conditions (Sjursen et al., 2011). At the pre-treatment examination, the mean number of amalgam surfaces was 23.5 (SD 10.6). The dental treatment was carried out by the patients' own dentists, who were instructed to follow written instructions from the Dental Biomaterials Adverse Reaction Unit regarding removal of amalgam restorations (Dental Biomaterials Adverse Reaction Unit, 2002). The dentists were instructed to use rubber dam, high-volume suction, water cooling and to remove fillings in chunks using a sharp dental bur. Only a few amalgam restorations should be removed at each treatment session.

Serum samples were collected at the pre-treatment examination before amalgam removal (baseline), and 3 and 12 months after the removal was finished. Samples of serum from twenty blood donors (6 men and 14 women, mean age 54 years, SD 6.4) matched for age and gender were retrieved from an existing biobank and used as comparison group (reference group).

All serum samples were stored at −80 °C before analysis. No patients had medication which was assessed to have influence on the serum levels of the analyzed cytokines.

The study was a part of a project approved by the Regional Committee for Medical Research Ethics in Western Norway (REK III, 24.01) and registered at ClinicalTrials.gov (NCT00346944). Changes of serum cytokine levels were not predefined as an outcome in the protocol of the main study.

2.2. Analyses

Cytokines in serum were analyzed using Luminex 100 system (Luminex Corp., Austin, TX, USA) (Heijmans-Antonissen et al., 2006). A multiplex bead kit (Human Cytokine Twenty-Five-Plex Antibody Bead Kit - LHC0009, lot 482849A, Invitrogen) was used. The kit includes four panels: An "inflammatory panel" (including GM-CSF, IL-1beta, IL-1RA, IL-6, CXCL8 (IL-8), and TNF-alpha), a "Th1/Th2 panel" (including IFN-gamma, IL-2, IL-2R, IL-4, IL-5, and IL-10), a "cytokine panel" (including IFN-alpha, IL-7, IL-12p40/p70, IL-13, IL-15, and IL-17), and a "chemokine panel" (including CCL11 (Eotaxin), CCL2 (MCP-1), MIG, CCL3 (MIP-1α), CCL4 (MIP-1β), IP-10 (CXCL-10), and RANTES). All samples were analyzed as single samples and in the same assay. One single plate was used in order to minimize analytical variability (Ellington et al., 2009). The cytokine detection limit varies individually depending on kit used and analyte. Typical lower level detection limit is in the range of 5-10 pg/ml. In our experiments we read a minimum of 100 beads ($n \geq 100$) per analyte (CV% is often below 10), and response data from the analyses are reduced to the median value before further statistical analysis. The quantification was performed using standard curves made by 5 parameter logistics of serial diluted standards.

Anti-nuclear antibodies (ANA) were analyzed by a bead-based multiplex immunoassay using QUANTA Plex™ SLE Profile 8 (Inova Diagnostics Inc., CATALOG #: 708910, San Diego, CA, USA). The assay was performed according to the manufacturer's instruction and provides semi-quantitative antibody levels against the following auto-antigens: Ro60 (SS-A), Ro52 (SS-A), La48 (SS-B), Jo1, Sm, RNP, Scl-70, Ribosomal P and Chromatin. The samples were read on a Luminex 100 (Luminex Corp., Austin, TX, USA) and analyzed using StarStation v.2.0 software (Applied Cytometry Systems, Dinnington, UK).

Concentration of mercury and silver in serum samples from the patient group and mercury in serum from the reference group was analyzed by sector field inductively coupled plasma–mass spectrometry (Rodushkin et al., 2004). Sample preparation was limited to a tenfold dilution with 2% HCl. Instrumental detection limits have been reported to 0.00085 μg/L for mercury and 0.00093 μg/L for silver (Rodushkin and Ödman, 2001). Actual limits of quantification (LOQ) in the analyses were 0.1 μg/L for mercury and 0.01 μg/L for silver.

2.3. Statistical methods

Instrument readings from the Luminex 100 system below the sensitivity (based on detectable signal >2 SD above background) given in the method documentation (Invitrogen Corporation, 2008) were treated as being below detection limit of the method and replaced by the sensitivity value divided by 2. Several variables had skewed distributions and some variables had many censored values due to concentrations below the detection limit. Thus, nonparametric tests were used. Mann–Whitney *U*-test was used for comparisons between the treatment group and the reference group. The Friedman test was used for comparisons of repeated measurements in the treatment group (baseline, and 3 and 12 months after removal of amalgam). This nonparametric procedure tests the null hypothesis of no significant differences between multiple responses over time. Significance was calculated using the module 'Exact Tests' in SPSS 15.0 (SPSS Inc., Chicago, IL). All tests were two-tailed and *p*-values less than 0.05 were considered significant.

3. Results

At baseline, the patient group had significantly higher values for IL-2R, IFN-alpha, IL-7, and IL-12p40/p70 compared with the reference group (Table 1). There were minor differences between the groups for GM-CSF and IL-6. Box-plots visualizing the distribution of these variables are given in Fig. 1. Overall, there were no significant differences of the values from the patient group over time. However, a minor, but statistical significant, decrease over time towards the median value of the reference group was found for GM-CSF, IL-8, and IL-7 (Table 1, Fig. 1). At baseline the median concentration of GM-CSF was 17 pg/ml. The median value 3 months after amalgam removal was unchanged, but at 1 year the median was below 15 pg/ml ($p = 0.041$; Friedman test). The concentration of IL-8 decreased from a median value of 17 pg/ml at baseline to 11 pg/ml and 13 pg/ml at 3 and 12 months, respectively ($p = 0.013$; Friedman test). For IL-7 the serum concentration was 49 pg/ml at baseline. Three months after amalgam removal the median value had decreased to 39 pg/ml, which was unchanged 1 year after removal ($p = 0.027$; Friedman test). Values for RANTES were not reliable due to sampling methodology, and thus, no data on RANTES are reported. Antinuclear antibodies (ANA) were detected in one patient. In the reference group no antinuclear antibodies were detected.

At baseline the mean mercury concentration in serum was similar in the patient group (1.00 μg Hg/L, SD 0.44) compared with the reference (0.97 μg Hg/L, SD 0.47). After amalgam removal the concentration of both mercury and silver in serum decreased significantly (Table 2).

4. Discussion

The hypothesis tested in this explorative study was that concentrations of a number of immune markers were similar before and after amalgam removal and that the levels were similar to a healthy reference group. The main finding was, however, that there were reductions of a few immune mediators in serum after removal of amalgam restorations. After removal of the amalgam restorations the values in the patient group were closer to the values of the healthy reference group (blood donors). Since a relatively large number of statistical tests were done and no adjustments for multiple comparisons of the *p*-values were done (Rothman, 1990),

Table 1
Concentration of cytokines in serum. Median concentration (pg/ml) in serum from patients before amalgam removal, and 3 months and 1 year after amalgam removal compared with data for the reference group.[a]

	Patients				Reference group	
	Before amalgam removal ($n=20$)	3 months after last removal session ($n=19$)	1 year after last removal session ($n=20$)	Significance for change over time ($n=19$)	Blood donors ($n=20$)	Significance for the difference between patients before amalgam removal and reference group
	Median	Median	Median	p-Value	Median	p-Value
Pro-inflammatory cytokines						
IL-1 beta	41	35	41	0.119	34	0.228
IL-6	26	24	25	0.597	22	0.031
IL-12 (p40/p70)	844	832	828	0.866	781	<0.001
IL-17	32	30	30	0.165	27	0.135
TNF-alpha	<10	<10	<10	0.667	<10	–
IFN-alpha	30	23	26	0.497	19	0.026
IFN-gamma	81	85	86	0.755	81	0.701
Anti-inflammatory/regulatory cytokines						
IL-1RA	718	487	510	0.078	464	0.248
IL-10	<5	<5	<5	0.395	<5	–
IL-13	<10	<10	11	0.995	<10	–
Immune modulatory cytokines and growth factors						
GM-CSF	17	17	<15	0.041	<15	0.036
IL-2	<6	7.8	8.8	0.956	<6	–
IL-2R	377	395	377	0.538	273	0.034
IL-4	132	133	128	0.331	132	0.673
IL-5	19	18	18	0.864	18	0.591
IL-7	49	39	39	0.027	39	0.024
IL-15	47	34	42	0.387	37	0.202
Chemokines						
MCP-1 (CCL2)	527	436	514	0.247	631	0.507
MIP-1α (CCL3)	70	66	66	0.621	54	0.089
MIP-1β (CCL4)	103	91	92	0.254	82	0.163
Eotaxin (CCL11)	170	151	174	0.543	189	0.185
IL-8 (CXCL8)	17	11	13	0.013	14	0.578
MIG (CXCL9)	88	64	89	0.470	51	0.103
IP-10 (CXCL-10)	86	82	82	0.207	77	0.050

[a] Concentrations below detection limit ("sensitivity"; based on detectable signal >2 SD above background) are indicated as "less than" (<) in the table.

it is not unlikely that some analyses showed statistical significant results due to chance. However, the results should be interpreted in the light of similar clinical studies (Pezelj-Ribaric et al., 2008) and data from other relevant studies (Gardner et al., 2009, 2010; Hemdan et al., 2007; Hultman et al., 1994, 1998; Pollard et al., 2001).

The finding of a decrease of IL-8 after the complete amalgam removal is interesting since subcytotoxic concentrations of mercury chloride stimulate the release of low levels of IL-8 by oral keratinocytes (Little et al., 2001). In addition, it has been reported that salivary concentration of IL-8 was reduced after removal of amalgam restorations in patients with oral lichenoid reactions related to their amalgam restorations and shifted towards levels found in healthy subjects (Pezelj-Ribaric et al., 2008). Since there is a correlation between concentration of IL-8 in saliva and in serum in patients with oral lichenoid reactions (Zhang et al., 2008) it seems likely that the reduction of IL-8 in serum was a result from the removal of the amalgam restorations even though the patients in the present study displayed no signs of oral lichenoid reactions. The level of IL-8 in the patient group was similar to the level in the reference group (Fig. 1) and comparisons with data from an amalgam free group could be useful in this context.

The pro-inflammatory chemokine IL-8 is released from several cell types in response to an inflammatory stimulus. It acts as a chemotactic factor attracting T-cells, basophils, and neutrophils, but not monocytes. IL-8 is also involved in neutrophil activation (Cammack et al., 2006). In the light of the reduction of health complaints in the patient group after removal of their amalgam restorations (Sjursen et al., 2011), it is interesting to note that IL-8 has been associated with pain in animal studies (Cunha et al., 1991). It has also been reported that patients with fibromyalgia had higher levels of IL-8 in serum than controls and it has been hypothesized IL-8 together with IL-6 may play a role in modulating fibromyalgia symptoms (Wallace et al., 2001). However, the reduction found in the present study was marginal and the finding may be without clinical relevance.

Table 2
Concentration of mercury and silver in serum. Concentration in serum from patients before amalgam removal, and 3 months and 1 year after amalgam removal.

	Before amalgam removal ($n=20$)	3 months after last removal session ($n=19$)	1 year after last removal session ($n=20$)	Significance for change over time ($n=19$) p-Value
Mercury (μg/L)				
Median	0.83	0.51	0.45	>0.001
Mean (SD)	1.00 (0.44)	0.51 (0.24)	0.54 (0.31)	
Silver (μg/L)				
Median	0.202	0.059	0.066	>0.001
Mean (SD)	0.263 (0.230)	0.081 (0.070)	0.085 (0.065)	

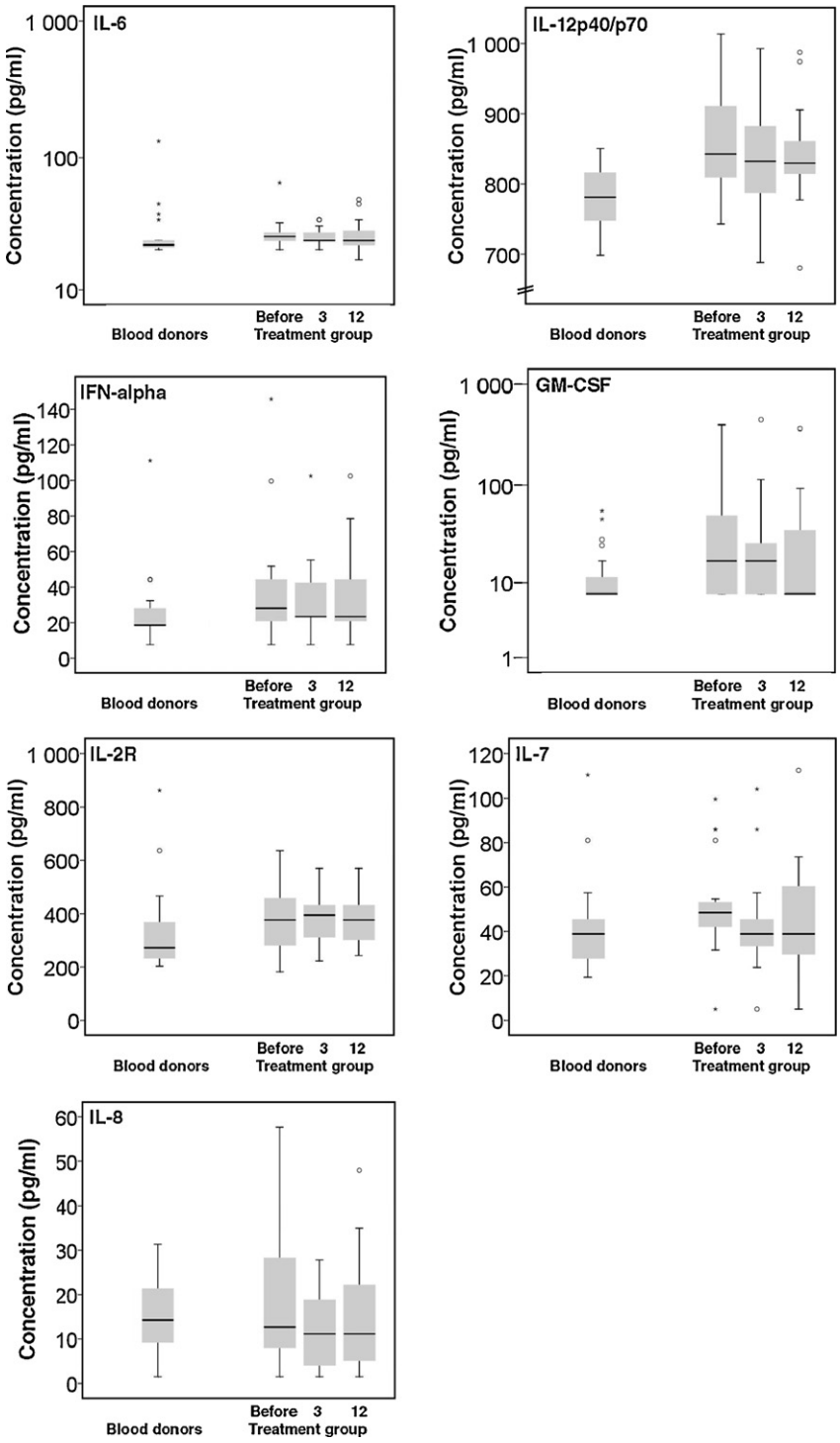

Fig. 1. Concentration of cytokines in serum. Concentration of IL-6, IL-12p40/p70, IFN-alpha, GM-CSF, IL-2R, IL-7, and IL-8 in serum in the reference group (blood donors) and in the treatment group before, and 3 and 12 months after complete amalgam removal. The box-plots summarize the median, upper and lower quartiles, and the maximum and minimum values. *p*-Values from statistical analyses are given in Table 1.

Not only concentration of IL-8, but also GM-CSF and IL-7 decreased after the amalgam removal towards the median value of the reference group. Both GM-CSF and IL-7 are growth factors. IL-7 up-regulates T cell differentiation (Murray et al., 1989) and the production of IL-8 (Standiford et al., 1992), while GM-CSF stimulates differentiation of granulocytes, macrophages, eosinophils and erythrocytes (Tarr, 1996).

In the patient group GM-CSF, IL-6, IFN-alpha, and IL-7 were higher at baseline than in the reference group, but after amalgam removal the levels were similar to the reference group. For IL-6 and IFN-alpha there were no significant change over time, and thus, the significant difference between the baseline value and the value for the reference group could likely be explained by chance. A potential effect associated with removal of amalgam on the levels of GM-CSF and IL-7 in serum needs to be verified in future studies.

IL-2R and IL-12p40/p70 were higher at baseline compared with the reference group and there was no significant change after amalgam removal on these markers. It could be speculated if there was

a general activation in the patient group of IL-2R and IL-12p40/p70 not related to amalgam exposure. In vitro experiments have shown that exposure of human immunocompetent cells to mercury chloride induced expression of the IL-2 receptor (Loftenius et al., 1997). However, since serum concentrations of IL-2R were unchanged after amalgam removal data from the present study do not support an association with exposure to amalgam. The higher levels of IL-12p40/p70 found in the patient group is interesting. IL-12 is a pro-inflammatory cytokine promoting Th1 cell differentiation and may stimulate secretion of both IL-8 and GM-CSF (Trinchieri, 2003). In a study on Hg-susceptible mice, it was shown that IL-12 down-regulated the autoimmune response to mercury and the production of antinucleolar antibodies (Bagenstose et al., 1998). Thus, it could be speculated about a protective effect from IL-12 regarding autoimmune response in the present patient group.

Both silver and mercury are released from amalgam restorations and absorbed (Björkman et al., 2007; Drasch et al., 1995; Lygre et al., 1999). It has been shown that mercury in serum correlates with number of amalgam surfaces (Bergdahl et al., 2012), and since concentrations of both mercury and silver in serum decreased after amalgam removal, concentration of mercury and silver in serum may be useful biomarkers for exposure to amalgam. Data on effects on the immune system in humans from these elements in combination are limited. Recently, it was reported that human peripheral blood mononuclear cells exposed in vitro to low concentrations of mercury released increased amounts of IL-1beta, TNF-alpha, IL-4, IL-17 and IFN-gamma and decreased amounts of IL1-RA and IL-10 (Gardner et al., 2009). Interestingly, the influence on the mercury mediated release of TNF-alpha from some background variables was tested and among several factors, significant effects were found for having no exposure to amalgam fillings (Gardner et al., 2009). A complicating factor is that the in vitro immune response, characterized by the Th1/Th2 balance, of human peripheral blood mononuclear cells after exposure to mercury may be depending on the type of activation used (Hemdan et al., 2007).

Minor effects on the immune system were reported for a group of individuals sensitized to mercury reflected by a reduction of the in vitro production of TNF-alpha and IL-1 (Langworth et al., 1993). Increased levels of antinuclear antibodies (ANA) have been reported to be associated with mercury exposure (Silva et al., 2004), but in the present study only one patient had detectable antinuclear antibodies and there were no indications of general autoimmunity in the patient group as reflected by ANA.

An increase of CD4+ lymphocytes in workers occupationally exposed to low doses of inorganic mercury has been reported suggesting an activation of the immune system (Alessio et al., 2002; Soleo et al., 2002). In the same group of workers a decrease of IL-8 in serum was found, suggesting an immunosuppressive effect from mercury. Interestingly, an inverse correlation between IL-8 in serum and mercury concentration in urine was found.

Ideally, samples from both controls and cases are taken simultaneously in parallel sessions, stored frozen and analyzed together in the same analysis. However, in this study the controls were matched by gender and age and samples were retrieved from an existing biobank of serum from blood donors. Because samples were not collected simultaneously from both cases and controls effects from storage cannot be excluded. However, since the levels of all but a few cytokines were similar between the groups, a systematic general effect from storage or sampling methodology is less probable.

There is a considerable variation between analytical results from multiplex bead kits from different manufacturers. Consequently, the absolute values of cytokine levels must be used with caution, especially when comparing cytokine levels from other manufacturers (Khan et al., 2004).

The clinical relevance of these findings is far from clear-cut at this stage, but data suggest a decreased Th1 response after amalgam removal, which may be related to the increased level of IL-12 in the patient group. Even though the complaint intensity may be reduced after amalgam removal (Sjursen et al., 2011), the complaints are not reduced to background levels found in the general population (Nerdrum et al., 2004). Thus, it could be hypothesized that the reduction of complaints could be mediated via a Th1 response facilitated by IL-12 in interaction with co-existing morbidity (Silbergeld et al., 2005).

The median concentration of IL-8 (CXCL8) was reduced from baseline to follow-up and at 3 months and 1 year. After removal of the fillings the median values were 65% and 76% of the median value at baseline and similar to the value in the reference group. Since cytokines act locally in tissues, the serum levels are only a reflection of the local production in the tissues. Although the levels in serum were moderately elevated, the local levels in saliva may be considerably elevated (Zhang et al., 2008). In the present patient group the intensity of general health complaints was higher than the intensity of local orofacial complaints (Sjursen et al., 2011). Therefore, cytokines in serum was used as a general marker of cytokine production in the body. The decrease of IL-8 in serum could be a normal response to the amalgam removal without any relation to health complaints in the patient group. Nevertheless, additional explorative studies are needed to investigate cytokine levels in patients with and without amalgam restorations and health complaints attributed to dental amalgam restorations since exposure to low levels of mercury may be a factor influencing cytokine expression (Gardner et al., 2009; Hemdan et al., 2007).

In summary, concentration of IL-2R and IL-12 was higher in the patient group and was not changed after the removal of amalgam restorations. This could be a result of a chronic activation of the immune system without relation to exposure to amalgam restorations. The decrease of IL-8 after removal of amalgam restorations towards the value in the group of blood donors may be related to the reduced amalgam exposure.

5. Conclusions

Data suggest that removal of amalgam restorations was associated with a decreased Th1-like response in a patient group with increased levels of IL-12. Even though the health complaint intensity was reduced in the patient group after amalgam removal, other factors than a decreased Th1-like response may be of importance for the improvement (Sjursen et al., 2011). Nevertheless, further studies on subjective health complaints including possible interactions between low level exposure to mercury and co-existing morbidity are warranted.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

Siren Hammer Østvold is gratefully acknowledged for technical assistance in relation to sample collection. Vigdis Helland is gratefully acknowledged for her excellent work with the coordination of the clinical examinations of patients. The project was funded by the Norwegian Ministry of Health and supported by the Centre for Clinical Dental Research at the Faculty of Medicine and Dentistry, University of Bergen, Norway.

References

Alessio, L., Apostoli, P., Cortesi, I., Lucchini, L., 2002. Synthesis of the results of studies performed on subjects exposed to low doses of mercury. Med. Lav. 93, 259–264.

Anneroth, G., Ericson, T., Johansson, I., Mornstad, H., Ryberg, M., Skoglund, A., Stegmayr, B., 1992. Comprehensive medical examination of a group of patients with alleged adverse effects from dental amalgams. Acta Odontol. Scand. 50, 101–111.

Bagenstose, L.M., Salgame, P., Monestier, M., 1998. IL-12 down-regulates autoantibody production in mercury-induced autoimmunity. J. Immunol. 160, 1612–1617.

Bergdahl, I.A., Ahlqwist, M., Barregard, L., Bjorkelund, C., Blomstrand, A., Skerfving, S., Sundh, V., Wennberg, M., Lissner, L., 2012. Mercury in serum predicts low risk of death and myocardial infarction in Gothenburg women. Int. Arch. Occup. Environ. Health, doi:10.1007/s00420-012-0746-8, Epub ahead of print.

Berglund, A., 1990. Estimation by a 24-hour study of the daily dose of intra-oral mercury vapor inhaled after release from dental amalgam. J. Dent. Res. 69, 1646–1651.

Björkman, L., Lind, B., 1992. Factors influencing mercury evaporation rate from dental amalgam fillings. Scand. J. Dent. Res. 100, 354–360.

Björkman, L., Lundekvam, B.F., Lægreid, T., Bertelsen, B.I., Morild, I., Lilleng, P., Lind, B., Palm, B., Vahter, M., 2007. Mercury in human brain, blood, muscle and toenails in relation to exposure: an autopsy study. Environ. Health 6, 30.

Cammack, R., Attwood, T.K., Smith, A.D., 2006. Oxford Dictionary of Biochemistry and Molecular Biology. Oxford University Press, Oxford, pp. XV, 720 s.

Cunha, F.Q., Lorenzetti, B.B., Poole, S., Ferreira, S.H., 1991. Interleukin-8 as a mediator of sympathetic pain. Br. J. Pharmacol. 104, 765–767.

Dental Biomaterials Adverse Reaction Unit, 2002. Fjerning av amalgamfyllinger (Removal of amalgam restorations). Nor Tannlegeforen Tid 112, 50–51.

Drasch, G., Gath, H.J., Heissler, E., Schupp, I., Roider, G., 1995. Silver concentrations in human tissues. Their dependence on dental amalgam and other factors. J. Trace Elem. Med. Biol. 9, 82–87.

Ellington, A.A., Kullo, I.J., Bailey, K.R., Klee, G.G., 2009. Measurement and quality control issues in multiplex protein assays: a case study. Clin. Chem. 55, 1092–1099.

Gardner, R.M., Nyland, J.F., Evans, S.L., Wang, S.B., Doyle, K.M., Crainiceanu, C.M., Silbergeld, E.K., 2009. Mercury induces an unopposed inflammatory response in human peripheral blood mononuclear cells in vitro. Environ. Health Perspect. 117, 1932–1938.

Gardner, R.M., Nyland, J.F., Silva, I.A., Ventura, A.M., de Souza, J.M., Silbergeld, E.K., 2010. Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. Environ. Res. 110, 345–354.

Gay, D.D., Cox, R.D., Reinhardt, J.W., 1979. Chewing releases mercury from fillings. Lancet 1, 985–986.

Heijmans-Antonissen, C., Wesseldijk, F., Munnikes, R.J., Huygen, F.J., van der Meijden, P., Hop, W.C., Hooijkaas, H., Zijlstra, F.J., 2006. Multiplex bead array assay for detection of 25 soluble cytokines in blister fluid of patients with complex regional pain syndrome type 1. Mediators Inflamm. 2006, 28398.

Hemdan, N.Y., Lehmann, I., Wichmann, G., Lehmann, J., Emmrich, F., Sack, U., 2007. Immunomodulation by mercuric chloride in vitro: application of different cell activation pathways. Clin. Exp. Immunol. 148, 325–337.

Hultman, P., Johansson, U., Turley, S.J., Lindh, U., Enestrom, S., Pollard, K.M., 1994. Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. FASEB J. 8, 1183–1190.

Hultman, P., Lindh, U., Horsted-Bindslev, P., 1998. Activation of the immune system and systemic immune-complex deposits in Brown Norway rats with dental amalgam restorations. J. Dent. Res. 77, 1415–1425.

Invitrogen Corporation, 2008. Human Cytokine Twenty-Five-Plex Antibody Bead Kit Information Sheet vol. 2011.

Issa, Y., Brunton, P.A., Glenny, A.M., Duxbury, A.J., 2004. Healing of oral lichenoid lesions after replacing amalgam restorations: a systematic review. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 98, 553–565.

Khan, S.S., Smith, M.S., Reda, D., Suffredini, A.F., McCoy Jr., J.P., 2004. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. Cytometry B Clin. Cytom. 61, 35–39.

Langworth, S., Björkman, L., Elinder, C.G., Järup, L., Savlin, P., 2002. Multidisciplinary examination of patients with illness attributed to dental fillings. J. Oral Rehabil. 29, 705–713.

Langworth, S., Elinder, C.G., Sundqvist, K.G., 1993. Minor effects of low exposure to inorganic mercury on the human immune system. Scand. J. Work. Environ. Health 19, 405–413.

Little, M.C., Watson, R.E., Pemberton, M.N., Griffiths, C.E., Thornhill, M.H., 2001. Activation of oral keratinocytes by mercuric chloride: relevance to dental amalgam-induced oral lichenoid reactions. Br. J. Dermatol. 144, 1024–1032.

Loftenius, A., Ekstrand, J., Moller, E., 1997. In vitro effects of mercuric chloride (HgCl2) on human mononuclear cells. Clin. Exp. Immunol. 110, 418–422.

Lygre, G.B., Gjerdet, N.R., Björkman, L., 2005. A follow-up study of patients with subjective symptoms related to dental materials. Community Dent. Oral Epidemiol. 33, 227–234.

Lygre, G.B., Høl, P.J., Eide, R., Isrenn, R., Gjerdet, N.R., 1999. Mercury and silver in saliva from subjects with symptoms self-related to amalgam fillings. Clin. Oral Investig. 3, 216–218.

Melchart, D., Vogt, S., Kohler, W., Streng, A., Weidenhammer, W., Kremers, L., Hickel, R., Felgenhauer, N., Zilker, T., Wuhr, E., Halbach, S., 2008. Treatment of health complaints attributed to amalgam. J. Dent. Res. 87, 349–353.

Murray, R., Suda, T., Wrighton, N., Lee, F., Zlotnik, A., 1989. IL-7 is a growth and maintenance factor for mature and immature thymocyte subsets. Int. Immunol. 1, 526–531.

Nerdrum, P., Malt, U.F., Høglend, P., Oppedal, B., Gundersen, R., Holte, M., Löne, J., 2004. A 7-year prospective quasi-experimental study of the effects of removing dental amalgam in 76 self-referred patients compared with 146 controls. J. Psychosom. Res. 57, 103–111 (comment in: J. Psychosom. Res. 2005; 2059: 2189–2090; author reply 2191–2002).

Nylander, M., Friberg, L., Lind, B., 1987. Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings. Swed. Dent. J. 11, 179–187.

Pezelj-Ribaric, S., Prpic, J., Miletic, I., Brumini, G., Soskic, M.S., Anic, I., 2008. Association between oral lichenoid reactions and amalgam restorations. J. Eur. Acad. Dermatol. Venereol. 22, 1163–1167.

Pollard, K.M., Pearson, D.L., Hultman, P., Deane, T.N., Lindh, U., Kono, D.H., 2001. Xenobiotic acceleration of idiopathic systemic autoimmunity in lupus-prone bxsb mice. Environ. Health Perspect. 109, 27–33.

Richardson, G.M., Wilson, R., Allard, D., Purtill, C., Douma, S., Graviere, J., 2011. Mercury exposure and risks from dental amalgam in the US population, post-2000. Sci. Total Environ. 409, 4257–4268.

Rodushkin, I., Engström, E., Stenberg, A., Baxter, D.C., 2004. Determination of low-abundance elements at ultra-trace levels in urine and serum by inductively coupled plasma–sector field mass spectrometry. Anal. Bioanal. Chem. 380, 247–257.

Rodushkin, I., Ödman, F., 2001. Assessment of the contamination from devices used for sampling and storage of whole blood and serum for element analysis. J. Trace Elem. Med. Biol. 15, 40–45.

Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1, 43–46.

Silbergeld, E.K., Silva, I.A., Nyland, J.F., 2005. Mercury and autoimmunity: implications for occupational and environmental health. Toxicol. Appl. Pharmacol. 207, 282–292.

Silva, I.A., Nyland, J.F., Gorman, A., Perisse, A., Ventura, A.M., Santos, E.C., Souza, J.M., Burek, C.L., Rose, N.R., Silbergeld, E.K., 2004. Mercury exposure, malaria, and serum antinuclear/antinucleolar antibodies in Amazon populations in Brazil: a cross-sectional study. Environ. Health 3, 11.

Sjursen, T.T., Lygre, G.B., Dalen, K., Helland, V., Lægreid, T., Svahn, J., Lundekvam, B.F., Björkman, L., 2011. Changes in health complaints after removal of amalgam fillings. J. Oral Rehabil. 38, 835–848.

Skare, I., Engqvist, A., 1994. Human exposure to mercury and silver released from dental amalgam restorations. Arch. Environ. Health 49, 384–394.

Soleo, L., Colosio, C., Alinovi, R., Guarneri, D., Russo, A., Lovreglio, P., Vimercati, L., Birindelli, S., Cortesi, I., Flore, C., Carta, P., Colombi, A., Parrinello, G., Ambrosi, L., 2002. Immunologic effects of exposure to low levels of inorganic mercury. Med. Lav. 93, 225–232.

Standiford, T.J., Strieter, R.M., Allen, R.M., Burdick, M.D., Kunkel, S.L., 1992. IL-7 up-regulates the expression of IL-8 from resting and stimulated human blood monocytes. J. Immunol. 149, 2035–2039.

Strömberg, R., Langworth, S., Söderman, E., 1999. Mercury inductions in persons with subjective symptoms alleged to dental amalgam fillings. Eur. J. Oral Sci. 107, 208–214.

Tarr, P.E., 1996. Granulocyte-macrophage colony-stimulating factor and the immune system. Med. Oncol. 13, 133–140.

Trinchieri, G., 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat. Rev. Immunol. 3, 133–146.

Vamnes, J.S., Lygre, G.B., Grønningsæter, A.G., Gjerdet, N.R., 2004. Four years of clinical experience with an adverse reaction unit for dental biomaterials. Community Dent. Oral Epidemiol. 32, 150–157.

Vimy, M.J., Lorscheider, F.L., 1985. Intra-oral air mercury released from dental amalgam. J. Dent. Res. 64, 1069–1071.

Wallace, D.J., Linker-Israeli, M., Hallegua, D., Silverman, S., Silver, D., Weisman, M.H., 2001. Cytokines play an aetiopathogenetic role in fibromyalgia: a hypothesis and pilot study. Rheumatology (Oxford) 40, 743–749.

Zhang, Y., Lin, M., Zhang, S., Wang, Z., Jiang, L., Shen, J., Bai, J., Gao, F., Zhou, M., Chen, Q., 2008. NF-kappaB-dependent cytokines in saliva and serum from patients with oral lichen planus: a study in an ethnic Chinese population. Cytokine 41, 144–149.