Cell Mediated Immune Affection Among Dental Staff Exposed to Metallic Mercury

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Abstract

For more than a century and half, silver/mercury amalgam fillings have been used in dental practice as the preferred tooth filling material. Currently, occupational exposure of dental staff to mercury vapor released from the amalgam, has been an issue of concern because of its possible adverse outcomes.

Aim: The aim of this work was to determine the mercury body burden in dental staff exposed to elemental mercury during their work course and the relation of this burden with exposure conditions. Besides, the study aimed at investigating the potential impact of metallic mercury vapor on the cellular immune system and cytokine (IL6) as a possible mechanism of its immunotoxicity.

Study Population: The study population consisted of group of dental staff (n=39) and a matched control group (n=42). Dental staff group was further subdivided into a group of dentists (n=21), and a group of nurses (n=18).

Methods: Each individual was subjected to detailed occupational and medical history taking and estimation of urinary mercury (U-Hg) and blood mercury (B-Hg) as indicators of mercury of body burden and exposure, respectively. Measurement of IL-6, CD3, CD4 and CD8 as immunological parameters.

Results: The study revealed statistically significant higher U-Hg and B-Hg levels in the dental staff compared to their controls. This elevation of mercury body burden was associated with marked significant reduction in CD3, CD4, and CD8 and increase in IL-6 among exposed group compared to the control group.

Recommendations: Exposure to mercury vapour produced in operating rooms is the main concern for dentists. Every effort should be made to avoid contact with mercury vapour if possible by using barrier techniques, reducing the temperature of the operating room and of the amalgam restoration. Air conditioning and proper ventilation of the operating room, the use of coolant sprays, good suction and proper handling of amalgam waste is recommended.

Key Words: Dental amalgam — Mercury vapor — Cell-mediated immunity — CD3 — CD4 — CD8 — IL-6.

Introduction

MERCURY is the only metal representing a volatile gas at room temperature, which is readily absorbed (80%) by the respiratory system. Mercury vapor from amalgam penetrate into tissues with great ease, because of its monopolar atomic configuration. Once inside the cells, mercury vapor is oxidized to Hg$^{2+}$, the very toxic form of mercury which binds covalently to thiol groups of proteins inhibiting their biological activity. Hg$^{2+}$ is more toxic than Pb$^{2+}$, Cadmium (Cd$^{2+}$) and other metals because it has a higher affinity due to “covalent bond” formation with thiol groups (cysteines in proteins) causing irreversible inhibition. Other metals form reversible bonds with proteins and are therefore less toxic [1].

Today the most widespread human exposures to mercury are, to ethyl-mercury as a preservative in vaccines, to methyl-mercury in edible tissues of fish, and to mercury vapor emitted from amalgam in tooth fillings [2].

Amalgam, which has been in use in dentistry for 150 years, consists of 50% elemental mercury and a mixture of silver, tin, copper and zinc [3].

SCENIHR (Scientific Committee on Emerging and Newly Identified Health Risks) state that “the incidence of reported adverse effects (in dental staff and dentists) is very low”.

Many researches revealed that dentists working with amalgam have an increased mercury exposure and this exposure resulted in significant adverse health effects [4]. Even 30 years after cessation of mercury exposure, dental nurses showed significant adverse health effects [5].

SCENIHR from the European Commission claim [6]: “Exposure to mercury is difficult to
measure. The indications for mercury exposure are therefore normally obtained by measuring mercury levels in urine and blood of individuals.

Exposure to mercury in dental personnel occurs during the placement of amalgam or composite fillings, the finishing and polishing of fillings, leaky amalgam capsules or malfunctioning bulk mercury dispensers, vaporization of mercury from contaminated instruments and the removal of old fillings. This can be through direct skin contact with mercury or freshly mixed dental amalgam or through inhalation of the mercury vapors.

Despite mercury exposure below safety limits “urine levels of mercury below 5 µg/l, which represent the No Observed Adverse Effect Level (NOAEL)”, significant adverse health effects were found in most studies in workers exposed occupationally to mercury, even several years after the exposure had ceased. It is impossible to determine any safety levels below which adverse effects can be excluded.

Studies have indicated that dental work involving mercury may be an occupational hazard with respect to reproductive processes, glioblastoma (brain cancer), renal function changes, allergies and immunotoxicological effects. Many studies have reported neurobehavioral changes and decreased performance in psychometric tests among dentists having elevated mercury levels in their urine samples.

Constant low-dose mercury exposure has been considered a possible cause for certain autoimmune diseases, e.g. multiple sclerosis, rheumatoid arthritis or systemic lupus erythematosus (SLE). These effects occur with exposure below mercury safety limits. Mercury compounds, especially metallic mercury, have immunity-stimulatory effects. Mercury can enhance humoral immunity, mainly antibody production by acting as a hapten or altering the antigenicity of cellular proteins thus causing hypersensitivity reactions mainly of types II and III. Contrary to immunity-stimulatory effects, recent research has shown that occupational exposure to inorganic mercury suppresses cell-mediated immunity and interferes with host defense against pathogens by direct action on cells of the immune system.

Aim of the work:

The first aim of this work was to determine the mercury body burden in dental staff exposed to elemental mercury during their work course and the relation of this burden with exposure conditions. Besides, the study aimed at investigating the potential impact of metallic mercury vapor on the cellular immune system and cytokine (IL6) as a possible mechanism of its immunotoxicity.

Subjects and Methods

Subjects:

The study population consisted of 81 males. The exposed group consisted of 39 dental staff (21 dentists & 18 dental nurses) working in the operative department of a dental school. The control subjects consisted of 42 individuals randomly selected from medical and nursing staff working in Kasr El-Aini Hospital. The age range of the individuals in the exposed group was (28-67 years) with a mean value of 43.23 ± 10.75 years showing no statistically significant difference when compared with the control group (range=25-66 years, mean=41.33 ± 10.78). As for sex, socioeconomic standards, amount of dental fillings in moths, and amount of fish consumption, the control subjects were selected so as to match the exposed subjects. The exposed subgroups (dentists and nurses) had matched mean values of age and working duration.

All dentists wear gloves regularly, but with irregular use of masks. The room of dental clinic is ventilated through windows. Dental nurses in this study did not use either masks or gloves during dealing with amalgams. The rooms of preparation are not well ventilated.

The control subjects were selected from medical and paramedical staff working in Kasr El-Aini Hospital. They were chosen as to be matched with the exposed personnel in age, sex, socioeconomic standards, amount of dental fillings in their Teeth, and amount of fish consumption. None of the control subjects had occupational history of exposure to any of mercury forms.

Methods:

All subjects underwent an interview including:

Full occupational history:

- Medical history emphasizing on the possible clinical manifestations of immune system disturbances.
- Laboratory investigations.
- Measurement of total mercury level in urine (U-Hg) and blood (B-Hg).
- Assessment of immune parameters through measuring (CD3, CD4, CD8 and IL6).
The counts of lymphocytes, (CD<sup>3+</sup>) T-cells, (CD<sup>4+</sup>) T-helper and (CD<sup>8+</sup>) T-suppressor in the peripheral blood of our studied group were assessed. The determination of T-cell populations was achieved through monoclonal antibodies which were used in indirect immunofluorescence tests.

The cytokine (IL6) was measured by enzyme-linked immunsorbent assay (ELISÂ) using a commercial kit (Quantikine, R&D Systems, Minneapolis, MN).

Statistical analysis:

Results were evaluated for each group. Data were compared using Student t-test. Analysis of variance (ANOVA) was used for multiple comparisons between the groups. Pearson correlation test was used to correlate between different variables among the exposed groups. The statistical significance was defined as p-value <0.05. Computer based statistical package for social sciences (SPSS) for windows 16 program was used.

Results

The results as shown as the Tables (1-5).

Table (1): Comparison between dental staff and control groups as regards different investigations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dental Staff N=39</th>
<th>Control N=42</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Hg µg/g creatinine</td>
<td>19.76±1.37</td>
<td>5.44±1.18</td>
<td>4.17</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>B-Hg µg/g creatinine</td>
<td>7.82±0.97</td>
<td>4.82±0.75</td>
<td>0.99</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD3</td>
<td>66.84±11.1</td>
<td>77.70±2.38</td>
<td>-7.47</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD4</td>
<td>42.10±9.21</td>
<td>54.33±5.52</td>
<td>-7.30</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD8</td>
<td>27.86±5.65</td>
<td>32.23±3.38</td>
<td>-4.24</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Statistically highly significant. ** Statistically significant.

Table (2): Analysis of variance (ANOVA) test of mean±SD of different investigations in dental staff subgroups (dentists and dental nurses) and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dentists N=21</th>
<th>Nurses N=18</th>
<th>Control N=42</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.85±14.11</td>
<td>46.30±3.02</td>
<td>41.33±10.78</td>
<td>1.44</td>
<td>n.s</td>
</tr>
<tr>
<td>Duration of work (in years)</td>
<td>18.14±12.12</td>
<td>21.66±3.83</td>
<td>1465.8</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>U-Hg µg/g creatinine</td>
<td>19.12±1.19</td>
<td>20.12±0.28</td>
<td>5.44±1.18</td>
<td>53.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>B-Hg µg/g creatinine</td>
<td>7.46±0.9</td>
<td>8.25±0.89</td>
<td>1.42±1.48</td>
<td>53.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.66±25.40</td>
<td>1.92±9.18</td>
<td>1.42±14.86</td>
<td>10.13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CD3</td>
<td>28.82±6.14</td>
<td>26.75±4.94</td>
<td>32.23±3.38</td>
<td>10.13</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* Statistically highly significant. ** Statistically significant.

Table (3): Multiple comparison using Post hoc test of mean±SD of different investigations in dental staff subgroups (dentists and dental nurses) and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dentists N=21</th>
<th>Nurses N=18</th>
<th>Control N=42</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Hg µg/g creatinine</td>
<td>19.12±1.19</td>
<td>20.12±0.28</td>
<td>&lt;0.05**</td>
<td></td>
</tr>
<tr>
<td>B-Hg µg/g creatinine</td>
<td>7.46±0.9</td>
<td>8.25±0.89</td>
<td>&lt;0.05**</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>1.66±25.40</td>
<td>1.92±9.18</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td>70.35±6.84</td>
<td>62.75±9.88</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>46.91±6.47</td>
<td>36.50±8.86</td>
<td>&lt;0.01*</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>28.82±6.14</td>
<td>26.75±4.94</td>
<td>n.s</td>
<td></td>
</tr>
</tbody>
</table>

* Statistically highly significant. ** Statistically significant.

Table (4): Correlation coefficient between duration of exposure to mercury and different investigations among exposed group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Hg µg/g creatinine</td>
<td>0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-Hg µg/g creatinine</td>
<td>0.51</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD3</td>
<td>-0.31</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD4</td>
<td>-0.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8</td>
<td>-0.29</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Table (5): Correlation coefficient between level of mercury in blood of the exposed group and different investigations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD3</td>
<td>-0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>-0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD8</td>
<td>-0.07</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>
Discussion

The property of mercury to amalgamate with other metals is used to create a material for filling teeth. This material remains the cheapest and most efficient in tooth restoration. Mercurial toxicity has been documented since.

Antiquity but the metal remains widely used in some countries [17]. Approximately 80% of inhaled elemental mercury is absorbed through the lungs by rapid diffusion, while dermal absorption represents about 2.6% as was estimated by Hursh et al., [18]. Although there is no ideal biological monitor for evaluating the risk of mercury intoxication from its metallic form, American Conference of Governmental Industrial Hygienists (ACGIH) (2003) [22], stated creatinine corrected urinary mercury (U-Hg) in spot urine samples, as the recommended biological monitor for workers exposed to metallic mercury with level of 1-5 µg Hg/g creatinine was determined as a background level in persons not occupationally exposed to mercury. The level of 35 µg Hg/g creatinine is considered as Biological Exposure Index (BEI) that necessitates exclusion of the mercury exposed worker to non exposed job till mercury levels decline to base values. Using U-Hg was adopted in many studies investigating mercury load in dental personnel. In the current study, the mean concentration of U-Hg in the dental personnel was statistically significant higher than that of their matched referents (19.76±1.37 versus 5.44±1.18, p<0.001). This goes in accordance with the results reported by Cianciola et al., [23] who found the mean levels of U-Hg in a group of dentists was 22.2 µg Hg/gm creatinine. Moreover, our results are greatly supported by the results of Karahalil et al., [24], and Ritchie et al., [25] as they reported U-Hg levels to be about 3-4 times more among dental personnel than that of their mercury unexposed controls.

As blood level of mercury reflects organic mercury as well as metallic and inorganic mercury (i.e. influenced by the consumption of fish contaminated methyl mercury), it is not recommended as reliable indicator of total body burden in longer term exposures [20]. It is useful primarily in cases of short-term, higher-level exposures to metallic form and a level of 15 µg/L is considered BEI [22].

Therefore, because our exposed subjects were exposed to amalgam on daily basis during their work course, it was not surprising having elevated blood mercury levels among the dental staff compared to controls (7.82±0.97 versus 4.82±0.75).

This goes in accordance with the results of many studies investigated blood mercury levels among dental staff [26] even and dental students [27].

Recently, Morton et al., [28] stated that dental workers remain an occupational cohort in whom the value of using different biological media to identify exposure to low level inorganic mercury can be investigated. The authors reported the mercury content in all biological material including fingernails, toenails, and pubic hair to be significantly higher in dental workers than in the control population. Thus, further comparison between dentists and dental nurses regarding (U-Hg) and (B-Hg) revealed statistically insignificant higher values (p>0.05) among the group of nurses. This difference can be attributed to the difference in exposure conditions such as wearing gloves and masks and the mercury levels in air with a consequent reflection on mercury levels in urine and blood. Similarly, in a Swedish study [29], dental nurses showed somewhat higher U-Hg than the dentists as measurements of mercury in the air of a dental clinic, with personal, active air samplers, showed a median air Hg of 1.8 µg/m³ for the dentists and 2.1 µg/m³ for the dental nurses.

A highly significant positive correlation was found between the duration of work and U-Hg among the group of dental staff (r=0.595, p<0.05). Similar positive correlation was reported by Ritche et al., [30]. This correlation can be explained by the fact that, urinary excretion increases from 13% to 58% after long-term exposure [20]. Although, B-Hg is not an indicator of total body burden, our results showed a positive significant correlation between duration of work and B-Hg (r=0.513 p<0.001). It was reported that mercury content in the blood is proportionately higher after a low dose than after a high dose, indicating that a higher proportion of the lower dose is oxidized as hydrogen peroxide-catalase pathway in red blood cells may be saturated at higher dose levels [21]. Mercury at very low (but non-toxic) levels can disrupt immune system homeostasis and may cause both clinical (autoimmunity, hypersensitivity) and subclinical effects (cellular and humoral immunologic variable modifications) [32]. Also, mercury was found to be able to suppress cell-mediated immunity [19]. Accordingly, as mercury can give rise to immunotoxic reactions which may be genetically regulated, in the absence of adequate dose-response studies for immunologically sensitive individuals, it has not been possible to set a level for mercury in blood or urine below which mercury related symptoms will not occur [31]. Our study illustrated
a statistically significant inhibition of cell mediated immunity represented by decreased CD3, CD4 and CD8 levels among exposed group especially among nurses compared to the control group and showed also a statistically significant increase of IL-6 among the exposed group. These findings are in accordance with the results obtained by Hemdan and co-workers [33].

Our study illustrated a statistically significant higher IL-6 among exposed workers than the control group, and this is in accordance with the results obtained with Podzimek and co-workers (2010) [34]. Who found increased production of IL-6 production with exposure to mercury, which is very often present in the form of amalgam in the oral cavity of persons in need of implant therapy, that can play an important role in immune reactions during implant healing process. In patients with failed titanium implants, decreased production of these cytokines may participate in implant failure.

Other researchers concluded that HgCl₂ stimulates IL-6 release from human mast cells. This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation [35].

In our research, we found that there is a statistically significant difference between the exposed and the control group as regards the cell mediated immunity in the form of CD3, CD4, and CD8 as we found a statistically significant reduction of the level of cells among the exposed group to metallic mercury than the control group. We found also a statistically significant correlation between the duration of exposure to mercury and the increase IL-6 and decreased D3, CD4 and CD8. Other researchers found that chronic exposure to mercury salts can lead to autoimmune like responses, mediated by autoreactive CD4+Th2 cells, that regulate and are followed by a resistant state mediated by autoreactive CD8+T cells, they report that exposure to mercury is long lasting and that regulatory CD8+Tc1 cells generated in tolerance are required to control the CD8-cell population from developing Th2-mediated autoimmunity [36].

We demonstrated in our research a statistically significant negative correlation between the blood mercury level and cell mediated immunity among the exposed group, and a positive correlation as IL-6. In accordance with our results Park et al., [37] found that numbers of CD4+CD8, CD45RA+ (suppressor-inducer) T lymphocytes and total CD4+T lymphocytes in the mercury workers were significantly smaller than those in the controls, and they concluded that T lymphocytes and CD57+CD16+NK cells are inversely affected by exposure to metallic mercury vapour in workers, with inversely correlated with urinary level of mercury.

**Recommendation:**

Exposure to mercury vapour produced in operating rooms is the main health hazard for dentists. Every effort should be made to avoid contact with mercury vapour if possible by using barrier techniques, reducing the temperature of the operating room and of the amalgam restoration. Air conditioning and proper ventilation of the operating room, the use of coolant sprays, good suction and proper handling of amalgam waste is recommended.

**References**