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# Effect of occupational exposure to elemental mercury in the amalgam on thymulin hormone production among dental staff

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Occupational exposure of dental staff to elemental mercury vapor released from dental amalgam is an issue of concern because of the possible immunological and neurological adverse outcomes. Recently, studies have reported that inorganic mercury induces immunosuppression by decreasing the production of thymus gland hormone (thymulin). This study aimed at investigating mercury body burden in dental staff and the relation of this burden to the potential impact of mercury on thymus gland hormone level (thymulin). Besides, the work aimed at verifying mercury effect on nitric oxide synthetase as a possible mechanism of its immunotoxicity. The study population consisted of a group of dental staff ( $n = 39$ ) [21 dentists and 18 nurses] and a matched control group ( $n = 42$ ). Each individual was subjected to detailed occupational and medical history taking and to estimation of urinary mercury (U-Hg) and blood mercury (B-Hg) as indicators of mercury body burden and exposure, respectively. Measurement of total thymulin hormone blood level, and plasma level of nitrite and nitrate (indicators of nitric oxide) was also done. The study showed a significantly increased U-Hg and B-Hg levels in the dental staff compared to their controls. This elevation of mercury body burden was associated with significant reduction in thymulin hormone blood level and nitric oxide parameters. These results were more evident in the group of nurses compared to the dentists. In conclusion, our results show that dentists and dental nurses have significant exposure to mercury vapor and point to the negative impact of mercury on thymus gland functions and confirm the implication that the nitric oxide pathway is a possible mechanism for this impact. Moreover, the study raises attention to the importance of hygiene measures in reduction of exposure to mercury vapor released from dental amalgam. *Toxicology and Industrial Health* 2009; **25**: 159–167.

**Key words:** cell-mediated immunity; dental amalgam; L-arginine nitric oxide pathway; mercury vapor; thymulin; thymus gland

## Introduction

For a century, mercury has been used in the dental practice as the preferred tooth filling for its capac-

ity of joining metals (amalgamate), low cost, rapid fixing in dental pieces, and reduced micro-leakage with time (ADA, 2003) However, because elemental mercury is absorbed through direct skin contact or inhalation, the use of mercury in dental amalgam continues to be a controversial issue, as it may pose occupational risks to dental practitioners and their assistants (Atesagaoglu, *et al.*, 2006).

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Mercury (Hg) has long been recognized as a neurotoxic metal. Many studies have reported neuro-behavioral changes and decreased performance in psychometric tests among dentists having elevated mercury level in their urine samples (Echeverria, *et al.*, 1995; Ritchie, *et al.*, 2002). Recently, some studies show mercury as immunotoxic although the mechanism responsible for its effect is not fully understood (Silbergeld, *et al.*, 2005). Mercury can enhance humoral immunity, mainly antibody production, by acting as a hapten or by altering the antigenicity of cellular proteins causing hypersensitivity reactions mainly of type II and III. Moreover, mercury has been linked to autoimmune disturbances (Kim, *et al.*, 2003).

In contrast to immunity-stimulatory effects of mercury, some studies reported that occupational exposure to inorganic mercury suppresses cell-mediated immunity and interferes with host defense against pathogens (Mozczyński, *et al.*, 1998). This immune-suppression may arise from the direct effect of mercury on thymus gland, which is the site where T lymphocytes differentiate from lymphoid stem cells, proliferate and mature into functional cells under the effect of its main hormone, thymulin (Savino and Darbenne, 2000). Valentino, *et al.* (2001) showed experimentally that mercury at very low concentrations has a toxic effect on thymic endocrine activity. The authors proposed inhibition of nitric oxide synthetase (NOS) enzyme as a suggested mechanism of the negative impact of mercury on thymus gland. Normally, NOS converts the cosubstrates L-arginine and O<sub>2</sub> into nitric oxide (NO), which has an important role in immune cells and thymus gland activity (Holán, *et al.*, 2002).

However, up to our MED LINE research, all the studies that showed the negative impact of inorganic mercury on thymus gland activity were of experimental nature. Therefore, we performed a cross-sectional study among a group of dental staff exposed to mercury during their daily work to determine the mercury body burden and to investigate the impact of mercury on thymus gland hormone level (thymulin). Both nitrites and nitrates were assessed to verify effect of mercury on L-arginine nitric oxide (NO) pathway as a possible mechanism of its immunotoxicity.

## Subjects and methods

### Participants

The study was conducted on all dental staff who were working in Pediatric Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University, Egypt (42 dentists, 19 dental nurses). The dental staff were asked to join our research; 12 refused and 10 were excluded according to the selection criteria, so the study included 39 personnel divided into 21 dentists (15 men and 6 women) and 18 dental nurses (all women). The selection criteria were that the dental personnel must have been working in dental services for at least the preceding 5 years and have worked with dental amalgam daily.

All dentists wore gloves regularly, but used masks occasionally. Rooms of the dental clinic were naturally ventilated through windows. Dental nurses never used protective equipment. The rooms for amalgam preparation were not well ventilated. The amalgam used was in the tablet form.

Every participant completed a questionnaire sheet including personal history, occupational history, and amount of fish consumption/week. Symptoms suggestive of possible clinical manifestations of immune system disturbances, namely repeated infections, arthralgias, hemolysis, falling of hair, skin diseases, allergies, were included in the questionnaire. Dental examination was done to determine the number of dental fillings.

The control subjects were selected from medical and nursing staff working in Kasr El-Aini hospital. They were chosen so as to match the exposed group as regards age, sex, socioeconomic standards, amount of dental fillings, and amount of fish consumption/week. None of the control subjects had occupational history of exposure to any form of mercury. Every control participant was subjected to the same questionnaire and dental examination as dental staff.

The nature of the study was fully explained to each studied personnel, accordingly, oral consents were obtained from every participant.

### Laboratory investigations

Blood was collected in metal-free polyethylene tubes. The blood samples were centrifuged at 1500 g for 20 min at 5 °C. Packed erythrocytes

and plasma were then separated. All the samples were stored at  $-80^{\circ}\text{C}$  until analyzed. Also urine samples (25 mL) were collected and stored at  $-80^{\circ}\text{C}$  until analyzed.

### 1. Blood urea and serum creatinine

### 2. Measurement of total mercury level in urine (U-Hg) and blood (B-Hg)

The samples were wet digested with nitric and perchloric acids (5:1) at  $25\text{--}35^{\circ}\text{C}$ . Then, the samples were filtered through Whatman Ashless Filter Paper and were added to bi-distilled water to the total amount of 10 mL. The mercury content was determined in wet-digested samples by a "cold vapor" atomic absorption technique using mercury evaporation kit (Varian 4S) at Varian 10plus AA. All chemicals used in the analysis were of the analytical grade. Calibration standard (CAL): a solution was prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration. Mercury Hollow Cathode Lamp (single element hollow cathode lamp or electrodeless discharge lamp and associated power supply) absorption cell (standard spectrophotometer cells 10-cm long, having quartz windows also) were used.

As for blood mercury, concentrations were estimated in  $\mu\text{g/L}$  and expressed as means of total mercury in blood, considering the plasma and erythrocyte ratio (2/3). As regards urinary mercury, it was corrected according to urinary creatinine and was expressed in  $\mu\text{g Hg/g creatinine}$  (Zimmer, *et al.*, 2002).

### 3. Assessment of total blood thymulin

Thymulin was detected in all samples by Enzyme Linked Immuno Sorbent Assay (ELISA) kit supplied by promokine bioscience D-69196 Heidelberg, Germany (Noureddin, *et al.*, 2006).

### 4. Nitric oxide indicators (plasma nitrites and nitrates)

They were measured by a method that depends on the fact that nitrite and nitrate form chromophore with Griess reagent and absorbance was read at 550 nm and sodium nitrite was used as a standard (Ding, *et al.*, 1988).

### Statistical analysis

Results were evaluated for each group. Data were compared using Student's *t*-test. Analysis of vari-

ance (ANOVA) was used for multiple comparisons between the groups. Pearson correlation test was used to test the correlation 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 Inc., Chicago, Illinois, USA) for windows 9.1 program was used.

## Results

Both exposed ( $n = 39$ ; 21 dentists and 18 dental nurses) and control groups ( $n = 42$ ) were matched. The age range of the individuals in the exposed group was 28–67 years with a mean value of  $43.23 \pm 10.75$  years showing no statistically significant difference when compared with the control group (range = 25–66 years, mean =  $41.33 \pm 10.78$ ;  $P > 0.05$ ). The amount of dental fillings (range = 0–7, median = 3 in exposed group and the range = 0–8, median = 4 in the control groups) showed no statistical difference of significance ( $P > 0.05$ ). The difference between the two groups was insignificant ( $P > 0.05$ ) as regards the amount of fish consumption (frequency/week, average amount, kind of fish).

Regarding the clinical manifestations among the study population (data not presented), none of the study groups showed manifestations suggestive of immune system disturbance. Evaluation of kidney functions was done to assess the effect of mercury. The results showed no abnormality in the renal function in both exposed and control groups, with no significant statistical difference between the mean values of blood urea and serum creatinine in both groups.

Measured creatinine-corrected urinary mercury (U-Hg) and total blood mercury (B-Hg) in the exposed workers were significantly higher than in the controls (Table 1;  $P < 0.001$ ). The mean value

**Table 1.** Mean  $\pm$  SD of total mercury in urine (U-Hg), total mercury in blood (B-Hg), total thymulin hormone, plasma nitrite and nitrate (indicators of nitric oxide) in dental staff and control groups

	Dental staff ( <i>N</i> = 39)	Control ( <i>N</i> = 42)	<i>P</i>
U-Hg, $\mu\text{g Hg/g creatinine}$	$19.76 \pm 1.37$	$5.44 \pm 1.18$	0.000*
B-Hg, $\mu\text{g/L}$	$7.82 \pm 0.97$	$4.82 \pm 0.75$	0.000*
Thymulin, $\text{Pg/mL}$	$0.48 \pm 0.16$	$0.80 \pm 0.14$	0.000*
Nitrite, $\mu\text{mol/L}$	$18.01 \pm 2.92$	$25.11 \pm 4.64$	0.000*
Nitrate, $\mu\text{mol/L}$	$7.44 \pm 2.28$	$9.14 \pm 1.88$	0.000*

\*Statistically highly significant.

**Table 2.** Mean  $\pm$  SD total thymulin hormone in different age groups of dental staff

	( $\leq 40$ years) <i>N</i> = 12	(41–49 years) <i>N</i> = 16	( $\geq 50$ years) <i>N</i> = 11	<i>P</i>
Thymulin, Pg/mL	0.512 $\pm$ 0.98	0.443 $\pm$ 0.16	0.5000 $\pm$ 0.20	n.s

n.s, Nonsignificant.

of total blood thymulin hormone was markedly lower among the dental workers than in the control subjects (Table 1;  $P < 0.001$ ). Similar decrements were recorded among the dental workers regarding plasma level of nitrites and nitrates, which were used as indicators of NO level. The level of nitrites and nitrates were higher in the control group with a difference of high statistical significance.

To investigate the effect of age and gender difference on the thymulin hormone level, the exposed dental workers were subdivided into three groups according to age  $\leq 40$  years ( $n = 12$ ), 41–49 years ( $n = 16$ ), and  $\geq 50$  ( $n = 11$ ). The mean  $\pm$  SD values of thymulin level among these groups showed no significant difference ( $P > 0.05$ , Table 2). Moreover, there was no statistically significant difference between male ( $n = 15$ ) and female ( $n = 24$ ) dental workers in the mean values of thymulin hormone

(0.450  $\pm$  0.14 and 0.49  $\pm$  0.17, respectively,  $P > 0.05$ ).

Further comparison between the exposed subgroups (dentists and nurses) and control group was done using analysis of variance test (ANOVA). The results showed statistically significant differences between the three groups in U-Hg, B-Hg, total thymulin, plasma nitrites ( $P < 0.001$ ), and nitrates ( $P < 0.05$ ; Table 3). Posthoc test (data not represented) showed higher mean values of U-Hg and B-Hg among the group of nurses than dentists (not statistically significant difference). However, both dentists and nurses had significantly higher U-Hg and B-Hg levels than control individuals. Total thymulin, plasma nitrites, and nitrates were significantly lower in the exposed subgroups compared to the control subjects. However, the last parameters were insignificantly lower in nurses than in dentists ( $P > 0.05$ ).

Correlation between different variables showed a positive significant correlation between duration of work and both U-Hg and B-Hg levels ( $P < 0.001$ ; Table 4). Total thymulin level and NO indicators (nitrites and nitrates) were negatively correlated with duration of work and age, with statistical significance only for nitrates (Table 4;  $P < 0.001$ ).

**Table 3.** Analysis of variance (ANOVA) test of mean  $\pm$  SD of age, duration of work, total mercury in urine (U-Hg), total mercury in blood (B-Hg), total thymulin hormone, plasma nitrite and nitrate (indicators of nitric oxide) in dental staff subgroups (dentists and dental nurses) and control groups

	Dentists ( <i>N</i> = 21)	Nurses ( <i>N</i> = 18)	Control ( <i>N</i> = 42)	<i>P</i>
Age	40.85 $\pm$ 14.11	46.30 $\pm$ 3.02	41.33 $\pm$ 10.78	n.s
Duration of work, years	18.14 $\pm$ 12.12	21.66 $\pm$ 3.83	—	n.s
U-Hg, $\mu\text{g Hg/g creatinine}$	19.12 $\pm$ 1.19	20.12 $\pm$ 0.28	5.44 $\pm$ 1.18	<0.001*
B-Hg, $\mu\text{g/L}$	7.46 $\pm$ 0.9	8.25 $\pm$ 0.89	4.82 $\pm$ 0.75	<0.001*
Thymulin, Pg/mL	0.49 $\pm$ 0.14	0.46 $\pm$ 0.18	0.80 $\pm$ 0.14	<0.001*
Nitrite, $\mu\text{mol/L}$	19.20 $\pm$ 2.04	16.62 $\pm$ 3.23	25.11 $\pm$ 4.64	<0.001*
Nitrate, $\mu\text{mol/L}$	7.67 $\pm$ 2.6	7.17 $\pm$ 1.73	9.14 $\pm$ 1.88	<0.05**

n.s, Nonsignificant.

\*Statistically highly significant.

\*\*Statistically significant.

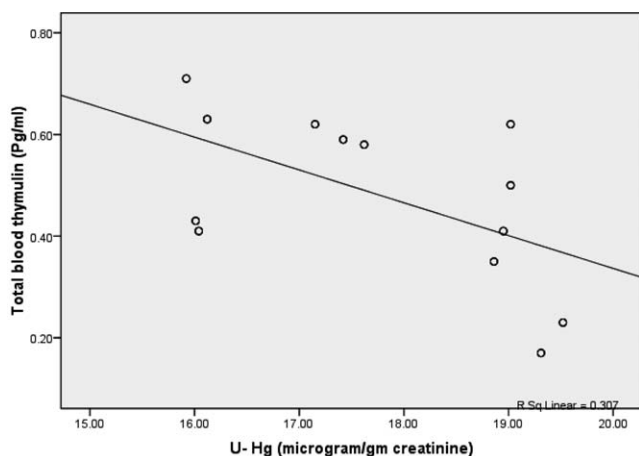
**Table 4.** Correlation coefficient between duration of work and age on one hand with U-Hg, thymulin hormone, nitrite, and nitrate in the dental staff group on the other hand

		U-Hg ( $\mu\text{g Hg/g creatinine}$ )	B-Hg ( $\mu\text{g/L}$ )	Thymulin (Pg/mL)	Nitrite ( $\mu\text{mol/L}$ )	Nitrate ( $\mu\text{mol/L}$ )
Age	<i>r</i>	—	—	-0.187	-0.193	-0.431
	<i>P</i>			0.254 (n.s)	0.240 (n.s)	0.006 (<0.05)*
Duration of work	<i>r</i>	0.595	0.513	-0.305	-0.27	-0.46
	<i>P</i>	<0.001	<0.001	n.s	n.s	0.003 (<0.05)*

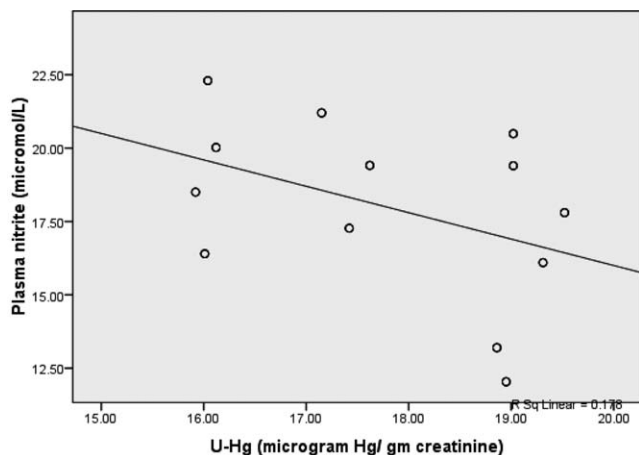
n.s, Nonsignificant.

\*Statistically significant.

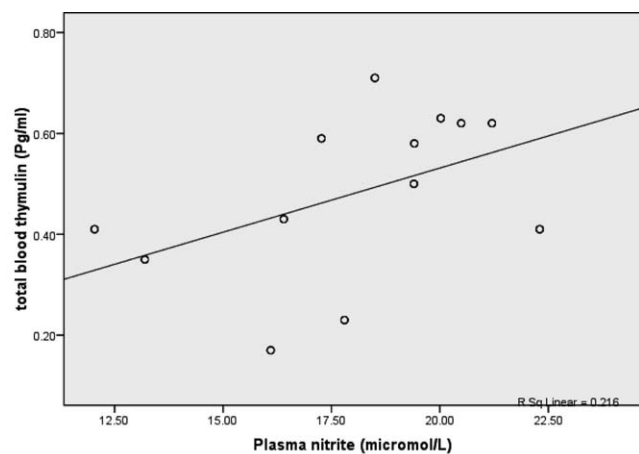




**Figure 1** Significant negative significant correlation between U-Hg and total blood thymulin in dental staff group ( $n = 9$ ) ( $r = 0.554$ ,  $P = 0.001$ ).



**Figure 2** Significant negative correlation between U-Hg and plasma nitrate in dental staff group ( $n = 39$ ) ( $r = 0.442$ ,  $P = 0.007$ ).



**Figure 3** Significant positive correlation between plasma nitrate and total blood thymulin in dental staff group ( $n = 39$ ) ( $r = 0.465$ ,  $P = 0.003$ ).

The correlations between U-Hg with thymulin level and plasma nitrite were presented in Figures 1 and 2 showing highly significant negative correlations. On the other hand, the relation between plasma nitrites and thymulin was positive with statistical significance (Figure 3).

## Discussion

This study shows that the total mercury body burden among the dental staff, particularly dental nurses, is significantly elevated compared with their controls. This elevation in mercury levels is accompanied by marked decrements in total blood thymulin level and NO indicators (nitrites and nitrates). This points to the inhibitory effect of mercury on NOS system resulting in decreased level of NO, which plays an important role in thymulin production.

There are many biomarkers of mercury exposure including mercury concentration in whole blood, plasma, urine, and hair. Nevertheless, there is no ideal biological monitor for evaluating the risk of metallic mercury intoxication (IPCS, 2000). However, the American Conference of Governmental Industrial Hygienists (ACGIH, 2003) set creatinine-corrected urinary mercury (U-Hg) in spot urine samples, as the recommended biological monitor for workers exposed to metallic mercury and the level of 1–5  $\mu\text{g Hg/g creatinine}$  was determined as a background level in persons not occupationally exposed to mercury. The level of 35  $\mu\text{g Hg/g creatinine}$  is considered as Biological Exposure Index (BEI) that necessitates exclusion of the mercury-exposed worker to another job where there is no mercury exposure till its level declines to baseline value. Therefore, using U-Hg was adopted in many studies investigating mercury load in dental personnel.

In our study, the remarkable increase in mean concentration of U-Hg in the dental personnel compared to their matched controls ( $19.76 \pm 1.37$  vs  $5.44 \pm 1.18$ ,  $P < 0.001$ ; Table 1) is consistent with other two similar Egyptian studies (El-Kholy, *et al.*, 1999; El-Olemy and Amin, 2003) that investigated the same parameter among dental staff. Moreover, our results are in accordance with the results reported by Rojas, *et al.* (2000) who found that the mean level of U-Hg in a group of

dentists was 22.2 µg Hg/g creatinine. Some investigators reported U-Hg levels to be about 3–4 times more among dental personnel than their unexposed controls (Karahalil, *et al.*, 2005; Ritchie, *et al.*, 2004).

As the blood mercury level reflects organic mercury as well as metallic and inorganic mercury (i.e., influenced by the consumption of fish contaminated with methyl mercury), it is not recommended as reliable indicator of total body burden in longer term exposures (IPCS, 2000). It is useful primarily in cases of short-term, higher level exposures to metallic form, and the level of 15 µg/L is considered the BEI (ACGIH, 2003).

However, because our study participants were exposed to amalgam on daily basis during their work, it was not surprising to have elevated blood mercury levels compared to their controls ( $7.82 \pm 0.97$  vs  $4.82 \pm 0.75$ ). This goes in accordance with the results of many studies that investigated blood mercury level among dental staff (Bake, *et al.*, 2002) and even dental students (Tezel, *et al.*, 2001).

Recently, Morton, *et al.* (2004) stated that dental workers remain an occupational cohort in which exposure to low-level inorganic mercury can be investigated. The authors reported that the mercury content in all biological samples including fingernails, toenails, and pubic hair to be significantly higher in dental workers than in the control population.

A “rough” correlation between levels of metallic mercury vapor in air and mercury levels in blood and urine was detected (ATSDR, 2003). However, in dentistry, the air Hg levels are mainly influenced by amalgam residues on the floor and by peak exposures during specific work tasks, such as preparation, insertion, and removal of amalgam fillings. Type of equipment, ventilation, floor covering, and the standards of hygiene (cleaning habits, handling of spills, etc.) are also important contributors to the degree of exposure (Langworthl, *et al.*, 1997). Unfortunately, environmental monitoring of mercury level in the dental clinic and the preparation room was not feasible in the current study but according to the work conditions, we can expect to find higher levels of mercury in air in the nurses’ preparation rooms where there is poor ventilation in addition to the fact that preparation of amalgam is carried out with higher chance for spills.

Thus, further comparison between dentists and dental nurses regarding U-Hg and B-Hg levels showed statistically insignificant higher values ( $P > 0.05$ ; Table 2) 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 (Langworthl, *et al.*, 1997), dental nurses showed somewhat higher U-Hg level than the dentists, which was related to measurements of mercury in the air of a dental clinic, using personal, active air samplers that showed a median air Hg of 1.8 µg/m<sup>3</sup> for the dentists and 2.1 µg/m<sup>3</sup> 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 Ritchie, *et al.* (1995), this can be explained by the fact that urinary excretion increases from 13 to 58% after long-term exposure (IPCS, 2000). Although B-Hg is not an indicator of total body burden, our results show 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 was proportionately higher after chronic low-dose exposure than after high-dose exposure (ACGIH, 2003).

The thymus gland is a central lymphoid organ in which bone marrow-derived T-cell precursors undergo differentiation and maturation into immunocompetent T cells (Anderson, *et al.*, 1996). These immunoregulatory functions of the thymus are accomplished through the classical thymic hormones thymulin, thymosins, thymopoietin and thymic humoral factor. Thymulin [Zinc-Factor Thymic Serum (ZnFTS)] is undoubtedly the best characterized of all thymic hormones (Darbenne and Savino, 1994). Thymulin forms with Th1 cells, IL-6, and natural killer cell cytotoxicity, a circuit that maintains the immunological homeostasis against toxic agents for the whole life of the organism. Failure of one component of the circuit leads to the frailty of the organism, which becomes an “immune low responder” with subsequent susceptibility to chronic inflammation and diseases (Santarrelli, *et al.*, 2006). In occupational settings, the mean plasma concentrations of active and total thymulin

were significantly lower in lead-exposed workers (Santarelli, *et al.*, 2005). In the context of mercury-induced immunosuppression, Valentino, *et al.* (2001) reported reduced thymulin hormone production and activity in mercury-exposed mice at different concentrations.

In this study, estimation of total thymulin levels among the dental workers showed significant reduction among the group of nurses than the dentists ( $0.46 \pm 0.18$ ,  $0.498 \pm 0.14$ , respectively) versus  $0.80 \pm 0.14$  in their controls ( $P < 0.001$ ). Moreover, thymulin level was inversely correlated with duration of work although this correlation did not reach the level of significance ( $r = -0.305$ ,  $P > 0.05$ ; Table 4). In fact, thymulin production declines gradually with aging (Consolini, *et al.*, 2000). This decline is probably due to impaired thymulin synthesis by thymus epithelial cells (TECs) or due to low availability of zinc ions inducing incomplete saturation and activation of all thymulin molecules produced by TECs (Mocchegiani, *et al.*, 2000). In the present study, the level of thymulin was declining with age but without significant difference ( $P > 0.05$ , Table 2). In addition, the correlation with age was negative but did not reach the level of significance ( $r = -0.187$ ,  $P = 0.254$ ; Table 4). However, the highly significant negative statistical association between U-Hg and B-Hg among dental staff in one hand and thymulin level on the other hand ( $r = -0.554$ ,  $-0.620$ , respectively) supports the hypothesis of the negative impact of mercury on the thymus gland and thymulin production. Recently, Santarelli, *et al.* (2006) reported that mercury induces significant reduction in TEC proliferation thus causing smaller number of thymulin molecules and reflecting a direct toxic effect on the biological functions of these cells. The reduction of thymulin level was independent of bioavailability of zinc although competition between zinc and mercury ions in saturating thymulin molecules may be present.

The molecular mechanisms by which mercury affects both thymic and peripheral immune efficiency are not fully understood. Loss of cell membrane integrity, inhibition of protein synthesis, and alteration of gene expression factors have been proposed to explain the toxic effect of mercury (Kim and Sharma, 2003). Recently, an inhibitory effect of mercury on L-arginine–nitric oxide (NO) pathway has been strongly added as one of the possible mechanisms by which mercury can exert its adverse

effects on the thymus gland (Santarelli, *et al.*, 2004). This inhibitory effect of mercury on NO pathway may be related to inhibition of NOS as shown for other metals such as lead, nickel, and cobalt (Tian and Lawrence, 1996).

NO was considered a toxic gas for more than two centuries but during the last 10 years, this belief was dramatically changed when several discoveries were made, revealing a significant biological role for NO (Roy, *et al.*, 2000). Any kind of infection (including bacteria and viruses) or cancer will lead to the production of cytokines. Cytokines carry the message from the infection site to the surrounding cells, which will start to induce NOS enzyme. The enzyme will continuously produce large amounts of NO for an extended period of time (several hours) at a sufficient concentration to locally inhibit DNA synthesis; hence, the profound cytostatic effect of NO on the proliferation of rapidly dividing tumor cells or pathogens (Holán, *et al.*, 2002).

Moreover, evidence from several studies suggest that NO is used within the thymus gland. Passage of lymphoid progenitors between the blood vascular supply and the thymic microenvironment is likely to be dependent upon NO as endothelium-derived relaxing factor (EDRF). Hence, NO may influence blood flow rates, cellular adhesion and therefore migration of blood-borne cells across vasculature within the thymus. Because NO has been implicated as a neurotransmitter (Tripathi, *et al.*, 2007), it may play a role as mediator of signals between intrinsic or projection neurons and cells of the thymus. Endocrine output of the thymus appears to be under the influence of NO (Downing, 1994).

As the average half-life of NO in tissue is about 3–6 s and in blood 1–2 s, NO is tested by measurement of nitrite and nitrate plasma levels (Pinto, *et al.*, 2003). In our study, marked decrement in nitrite and nitrate was detected among dental personnel particularly in dental nurses. The decrement of both indicators was significantly inversely related to duration of work, U-Hg and B-Hg on one hand and positively significantly correlated with thymulin level on the other. This reinforces the hypothesis of the negative impact of mercury on NOS enzyme. In accordance to our findings, Kim, *et al.* (2002) reported decreased levels of nitrite and nitrate among a group of mice



experimentally exposed to mercury. In another study, this negative impact was reversed by adding arginine amino acid as a diet supplement to mice receiving inorganic mercury in diet. This finding shows the protective role of arginine on thymic endocrine efficiency and suggests that daily low arginine intake may account for individual susceptibility to mercury-induced immunological effects, which were detected in mercury-exposed workers (Santarelli, *et al.*, 2004).

## Conclusions and recommendations

This study showed that dentists and dental nurses have increased body burden of mercury as revealed from the high U-Hg and B-Hg compared to their controls. Although these levels were below BEI stated by OSHA and ACGIH, the dental personnel experienced a condition of subclinical immunosuppression as evident from the marked depression in thymulin hormone level.

Our study confirms other studies claiming that mercury exerts its immunotoxic effects through inhibition of NOS enzymes. This inhibition results in reduction of NO, which has direct cytotoxic effect on pathogens in addition to its role in thymulin hormone production. However, further larger scale human studies are needed for more clarification of this issue.

As elemental mercury is an indispensable material in oral amalgam, there is an intense need to better training of all personnel involved in the handling of mercury and dental amalgam regarding the potential hazards of mercury vapor and the necessity of observing good mercury hygiene practices.

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