ORIGINAL RESEARCH

Hair Mercury Levels in Periodontal Patients in Comparison with Healthy Individuals

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ABSTRACT

Introduction: The clinical manifestation of periodontal diseases (such as gingivitis and chronic periodontitis) results from a complex interplay between the etiologic agents such as bacteria that present in the dental plaque, genetic factors, systemic diseases, smoking and exposure of some heavy metals, such as mercury. In this study, we aimed to evaluate hair mercury levels in healthy subjects in comparison with periodontal patients.

Materials and methods: One hundred twenty subjects were enrolled in this study. The included persons were divided into 3 groups: healthy subjects (n = 40), gingivitis (n = 40) and chronic periodontitis patients (n = 40). Hair samples were collected from occipital area of head. Total mercury levels were determined by atomic absorption spectrophotometry.

Results: The difference between mercury levels in three groups were statistically significant (p-value < 0.001). Mercury level in periodontitis patients was greater than the gingivitis group (p-value < 0.001). In addition the differences between mercury levels in periodontitis patients *vs* healthy individuals was significant (p-value = 0.048). The gingivitis patients had lower levels of mercury than the control group, but the difference was not significant (p-value = 0.170).

Conclusion: The results showed that the levels of mercury are to some extent differed in periodontal diseases in comparison with the healthy individuals. A study with larger sample size is needed for clarification of this issue.

Keywords: Chronic periodontitis, Gingivitis, Mercury.

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INTRODUCTION

Periodontitis is an inflammatory diseases that affecting the periodontium. Loss of the alveolar bone, loosening and loss of teeth are the major problems that accompanying in this disease. This disease is classified to the following major categories, i.e. gingivitis, chronic periodontitis, aggressive periodontitis, periodontitis as a manifestation of systemic disease, necrotizing ulcerative gingivitis/periodontitis, abscesses of the periodontium and combined periodontic-endodontic lesions.[1]

There are various factors that are related to the etiology of this disease. It is proposed that poor or ineffective oral hygiene is located in the first line. This leads to the formation of dental plaque that is consisted of mycotic and bacterial matrix. Malnutrition, some systematic diseases, such as diabetes and smoking are the other important causes of periodontitis.[2-12]

There are evidences that heavy metal exposure, such as mercury and its derivatives are connected to the oral health conditions and diseases.[12] Today the most widespread exposures to mercury are from three ways: Mercury vapor, methylmercury in sea foods and ethylmercury in the form of thimerosal used as a preservative in vaccines.[13] After exposure to the mercury and its derivatives, such as methylmercury, bulk of them accumulates in human hair.[14] Contamination of sea foods to mercury is a major route of mercury entrance to human body. In addition, there are other ways in which mercury can enter to the body. Use of amalgam as a dental restorative material, in which mercury is one of the important constituents, is another route.

In this case control study, we aimed to determine the levels of human hair contamination with mercury in periodontitis patients. To end this, we measured the hair levels of mercury in healthy individuals in comparison of periodontitis patients.

MATERIALS AND METHODS

Study Population

One hundred twenty subjects were participated in this study. The subjects were selected among outpatients that referred to the Periodontics Department of Dentistry Faculty in Babol University of Medical Sciences. Subjects were classified into

3 groups: Group A was the healthy individuals without any sign of periodental diseases. Group B was the persons with gingivitis and group C consisted of chronic periodontitis patients. Diagnosis of gingivitis and chronic periodontitis were done according to the standard criteria as follow:

- *Gingivitis*: Bleeding from the gingival sulcus on gentle probing and color change of gingiva from pink to red or bluish red color and a ginigival index <1.
- *Chronic periodontitis*: Pocket formation that was deeper than 4 mm with clinical attachment loss that was greater than 2 mm.[15]

Each person with a history of cigarette smoking, systemic disorders (such as diabetes, rheumatoid arthritis, genetic disorders, psychosocial disorders and cardiovascular disease) and any history of periodontal treatment during the last 6 months were excluded.

Hair Sampling and Analyses

After receiving the written consent from each person, hair strands from the root were taken from the occipital area of head with stainless steel tweezer. Hair were placed into a capped tube and sent to the biochemistry and biophysics department of medicine faculty.

Digestion Procedure

For measurement of total mercury in human hair, sample preparation and digestion were conducted as reported in previous works with some modification.[16] The hair samples were cut in very small pieces and ultrasonically (Bandelin Sonopuls, Germany) washed in a 0.1% Triton X-100 solution for 20 mm in department of biochemistry and biophysics. After rinsing the samples with deionized water, hair samples were rinsed with acetone and air-dried. Nearly 40 mg of the dried hair was placed in a 5 ml test tube and 700 μl of HNO_3 (5M) was added to the samples. Then tubes were heated at 100°C for 120 minutes. Digested samples were stored and protected from light, at 4°C for next procedure.

Extraction Procedure

After cooling down the digested samples, 4 ml of NaOH 1 M and 1 ml of acetate buffer solution were added to the sample in order to obtain a pH of 4.5. 200 μl of the 1% ammonium pyrrolidine dithiocarbamate (APDC) solution and 500 μl of methyl-isobutylketone (MIBK) were the other materials that added to the samples. Samples were vigorously mixed and centrifuged at 2700 RPM for 20 minutes. Finally, a 20 μl of the supernatant organic phase containing the Hg-APDC complex was directly introduced into a graphite tube of atomic absorption spectrophotometry (AAS) and atomized.[16]

Analysis of the Mercury Levels by AAS

PG990 atomic absorption spectrophotometer equipped with graphite furnace was used for measurement of hair mercury levels. The operating parameters were set as recommended by manufacturer. A stock standard solution of mercury (Hg) at a concentration of 1000 mg/l (ppm) was used for drawing the standard curve. The calibration curve was established using the following working standards solutions: 0, 7.81, 15.62, 31.25 and 62.50 μg/l (ppb). The thermal cycle of the AAS program for measurement of hair mercury levels are presented in Table 1.

Statistical Analysis

Results of the numerical variables are presents as mean ± SD. A p-value less than 0.05 (two tailed) considered significant. For comparison of various numerical variables, we used from ANOVA test, Scheffe post hoc model. All statistical analysis was done by commercially available program of SPSS version 18.

RESULTS

In this study, 120 subjects were enrolled and classified into 3 groups: healthy subjects, gingivitis patients and chronic periodontitis patients. Characteristics of the included persons are presented in Table 2. In addition in this table hair mercury levels in included persons are also presented. As we observe in this table, chronic periodontitis patients had the highest levels of mercury levels in their hair, but the gingivitis patients had the lowest level of mercury. For comparison of mercury levels in our included groups, simultaneously,

Table 1: Atomic absorption spectrophotometry working condition for total mercury determination

Step	Temperature (°C)	Ramp (°C/S)	Hold time	Argon flow rate (ml/min)
Drying	120	205	15	300
Pyrolysis	250	15	20	300
Atomization	1600	1	4	Off
Cleaning	2000	1	2	300

Table 2: Characteristics of the included persons in study

Disease/ variables	Healthy persons	Gingivitis patients	Chronic periodontitis patients
Age (years)	25.5	31.2	41.1
BMI (kg/m²)	23.9	25.1	27.6
CAL (mm)	—	—	>2
GI	<1	>1	—
Mercury levels (ppb)	12.7 ± 8.0	9.5 ± 5.1	16.9 ± 8.5

BMI: Body mass index; CAL: Clinical attachment loss; GI: Gingival index

Table 3: Mercury levels in included persons according to the Age and BMI subclassification

Disease/ variable	Age subclassification			p-value	BMI subclassification			p-value
	<30	*30-40*	*>40*		*19-25*	*25-30*	*>30*	
Healthy persons	12.65 ± 7.7	13.70 ± 10.9	*—	0.791	11.28 ± 7.6	16.93 ± 7.9	12.05 ± 8.5	0.323
Gingivitis patients	9.15 ± 5.5	9.38 ± 4.0	12.27 ± 6.8	0.552	8.6 ± 4.3	11.6 ± 6.1	6.46 ± 3.5	0.134
Chronic periodontitis patients	10.50 ± 5.9	17.21 ± 7.5	17.41 ± 9.5	0.560	17.14 ± 10.4	17.80 ± 7.8	14.42 ± 9.1	0.641
p-value	0.206	0.018	0.321	—	0.016	0.052	0.397	—

*There was no person in this age category

ANOVA test were used. The differences in the mercury levels in three groups were statistically significant ($p < 0.001$). The differences between mercury levels in healthy person's *vs* gingivitis patients were not significant (p-value = 0.170), but with periodontitis patients were significant (p-value = 0.048). In addition we observed that, the differences between mercury levels in gingivitis patients *vs* periodontitis patients were significant (p-value < 0.001).

For evaluation of the probable impact of age and BMI on the levels of mercury, we subclassified the age and BMI variables as follow:

Age: Age < 30, 30-40 and >40 years old.

BMI: 19 < BMI < 25, 25 < BMI < 30 and BMI > 30 kg/m^2.

The mercury levels in these subclassification groups are presented in Table 3. Chronic periodontitis patients with lower than 30 years old, had lower mercury levels and patients with greater than 40 years old, had the highest hair mercury levels. Again the levels of mercury in gingivitis patients were the lowest, but with increasing the age an increase in the levels of mercury were observed. Such trends were observed in chronic periodentitis patients and also healthy individuals. Our finding related to the impact of various BMI classes on hair mercury levels are also presented in this table. It seems that the impact of BMI on hair mercury levels is not in a definite trend.

DISCUSSION

For analysis of the levels of mercury in human body, several indicators such as blood,[17] urine and hair[18] were introduced. It is proved that hair is a suitable tissue for indication of human exposure to mercury.[16] In this study we used from subjects hair as a source of probable contamination with mercury. The determination of mercury in hair is a time consuming procedure but a precise method. For analysis of total mercury in hair, digestion with HNO_3 instead of HCl or H_2SO_4 is preferred.[16] In addition for detection of the mercury levels we used graphite furnace atomic absorption spectrophotometry that is a powerful tool that able to detect the levels of elements in ppb scale.

According to the result of the present study, chronic periodontitis patients had the highest level of mercury in comparison with the gingivitis and healthy individuals. As previously mentioned it, exposure to mercury in form of inhalation or by foods especially sea foods, are known as one of the risk factors of oral and mouth diseases. In our study population, the levels of mercury were not in toxic range,[19] but chronic periodontitis patients had the highest mercury levels. In addition there is a hypothesis that stated with increasing in the age, there is a gradual increase in human mercury levels.[20] In the present study, data showed that subjects with higher age had higher hair mercury levels in all groups. This emphasizes that with aging an increase in hair mercury levels occurs.

For evaluation of the probable impact of BMI on the levels of mercury, analysis of the hair mercury levels, according to the BMI subclassification were performed.[21] We observed that persons in BMI of 19 to 25 kg/m^2 had a statistically significant difference in their hair mercury levels and again the chronic periodontitis patients had the highest level.

The reported studies regarding to the hair mercury levels in oral and mouth problems is very low. In a study that carried out by Han et al on 598 males and 730 females in Korea, researchers showed that mercury exposure is associated with periodontitis (odds ratio = 3.17) and males had higher probability of having periodontitis than females.[19] Dental health of 73 workers previously exposed to mercury vapor was compared with 51 nonexposed individuals by Holland et al. Researchers did not find a correlation between mercury exposure and periodontal disease and reported no significant difference between the exposed workers and the referents.[22] Kim et al in 2013 studied the levels of mercury in blood (but not hair) of periodontitis patients. They observed that association between blood mercury levels and periodontitis was significant in the Korean male population.[23]

Various mechanisms are proposed for possible role of mercury in creating the oral and mouth problems. It seems that after mercury exposure, impairment of lymphocyte proliferation, cytogenetic damage and incidence of acute and chronic inflammation are the major probable mechanisms.[24]

This study had some limitations. First of all, the patients were not matched according to the number of filled teeth with amalgam. According to the evidence-based analysis, an amalgam restoration is safe[25] and some studies did not find a correlation between hair mercury levels with number of dental amalgam fillings.[26] In addition it was better that the subjects matched according to their diet, because one of the other major route of mercury entrance to the body is foods especially sea foods. It seems that another study with the mentioned criteria's is needed to reach to an exact conclusive statement for the potential role of mercury as a periodontitis inducing agent in our population.

CONCLUSION

It seems that the level of hair mercury levels in chronic periodentitis patients is higher than the healthy individuals. It is needed to more comprehensive study for reaching to an exact conclusive statement for the potential role of mercury as a periodontitis inducing agent in our population.

ACKNOWLEDGMENTS

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