

The Assessment of Mercury Released from Dental Amalgams after Exposure to Wi-Fi and X-Ray Radiation in Artificial Saliva

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Received 20 August 2017 • Revised 29 October 2017 • Accepted 27 December 2017

ABSTRACT

Mercury is a well-known toxic element that is found in various forms in nature. Recently, dental amalgams have been recognized as a new source of mercury. This study was carried out under in vitro condition; the amount of mercury released from teeth restored with amalgams in the presence of Wi-Fi router radiation (non-ionizing radiation) and X-ray (ionizing radiation) using a protocol similar to Computed Tomography of Para-nasal Sinuses (CT) were analyzed separately, and in combination on the samples. For this reason, 50 human premolars were restored with a certain type of commercial amalgams. The samples were divided into five groups; control, only-CT, CT+Wi-Fi, Wi-Fi+CT and only-Wi-Fi groups, and mercury measurement were investigated at 24 and 48 hours after exposure to radiation by cold-vapor atomic absorption system. The results showed a significant difference between subgroups 24 and 48 h (p-values = 0.001 and 0.008). However, over time a significant difference was only found in CT+Wi-Fi group (p-value = 0.043). In addition, in comparison with the control group, only the subgroup that was exposed to Wi-Fi waves showed a significant difference after 24 hours (p = 0.033). In line with some of the previous studies, our findings showed that electromagnetic waves are involved in mercury release process, and warnings about the consequences of electromagnetic waves on mercury release and substituting it with a new compound in restorative dentistry should be taken into consideration.

Keywords: mercury toxicity, dental amalgam, mercury release, Wi-Fi waves, X-ray, PNS

INTRODUCTION

Mercury is one of the few metals discovered by mankind, which is in the form of liquid under the standard conditions (temperature and pressure), found in various forms [1]. It has many applications such as in thermometers, barometers, batteries, fluorescent lamps, mercury switches, and some insecticides [2]. In addition, mercury can be mixed with almost all metals, including silver and gold to create alloys, called amalgam [3]. Dental amalgams are one of the oldest most consumables in restorative dentistry [4]. Furthermore, it seems that there isn't yet another more cost-effective option to be used as a replacement [5].

In one hand, various dangers are caused by mercury in the vicinity of living organisms and biological environments, known as mercury toxicity [6]. Dental amalgams are known as a modern face of mercury toxicity

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along with ethyl and methyl forms [7]. One of the most horrible threats of mercury is its concentration in the brain tissues, and its hazardous effects on the nervous system, and its trace has been found in many diseases such as Alzheimer's [8] and Parkinson's disease, and more recently heart failure [9-10]. Also, harmful effects of mercury, especially through restorative amalgam on the health of children at different age levels, and pregnant and lactating mothers in particular have been seriously taken into consideration and warned by scholars [11-13]. Generally, it is not extolled if we say that exposure to mercury should be considered a silent threat to life and the environment [14]. However, what has attracted the attention of scholars concerning the applications of amalgams is the toxicity, caused by the release of mercury. Although it was initially thought that amalgams seem to be safe, [15] it has been shown that mercury released in amalgams in forms of fine particles can be either inhaled or swallowed. Consequently, its adverse health effects have become a serious and ongoing debate in recent years [7, 16].

On the other hand, there has been a boom in the use of communication devices such as cell phones, Wi-Fi routers, laptops, radars and etc... that use microwave signals for communication. These signals as a part of the continuous spectrum of electromagnetic waves are non-ionizing radiations, and for a while only their thermal effects were taken into consideration to be evaluated for their adverse effects [17-18].

Another part of the electromagnetic spectrum is ionizing radiations such as X- and gamma rays. Although such radiations have been used in different filed of sciences, including radiology for diagnosis and even treatment, they have more known effects due to their longer history of usage than non-ionizing radiations [19]. What researchers have come to notice is that these electromagnetic waves can affect the mercury release rate from dental amalgams.

In the early studies, it was reported that divers who were conducting electrical welding and cutting underwater complained about a metallic taste in their mouths [20]. In the same year, it was shown that some imaging monitors could release mercury in the amalgams [21]. In other research finding, the mercury released from dental amalgams was significant in patients' saliva and urine samples after being exposed to magnetic MRI fields (0.23T), and mobile devices [22]. The effect of magnetic fields emitted from MRI with identical conditions on teeth restored with different commercial amalgams showed different microleakages of mercury in vitro conditions (where human teeth were studied in artificial saliva) [23]. Briefly after, the role of MRI irradiation (1.5T) in the release of mercury in the urine of students with restored teeth was reported [24]. In vitro study on restored teeth in the presence of MRI (1.5T) and X-rays was performed, separately, and it was stated that despite the presence of mercury in saliva in the presence of X-rays, there was no specific MRI effect [25]. However, in the criticism of this report, the absence of MRI effects was attributed to methodological errors [26]. Finally, the results of radiation from Wi-Fi devices on treated teeth in in-vitro conditions showed that these waves accelerated the release of mercury [27].

In this study, the release of mercury from amalgam-treated teeth in the presence of irradiation from Wi-Fi and X-ray devices under conventional conditions of Computed Tomography of the Para-nasal Sinuses (CT) was performed separately, and in combination with different sequences. This study was performed in in-vitro conditions. To the best of our knowledge, this study is the first attempt that has explored combination of radiation on amalgam.

MATERIAL AND METHODS

Preparation of Restored Samples

After obtaining permission from the Ethics Committee of Shiraz University of Medical Sciences, dental samples restored with amalgams were prepared. In this in vitro study, 50 premolar teeth that were intact and similar in appearance were collected from orthodontic clinics. In order to create an equalized condition and minimize interventional factors taken into account from the previous studies, infections were initially removed from the teeth collected by an autoclave; Class-V cavities with the same dimensions (5 mm mesodestinally, 3 mm ecosoaginally, and an axillary depth of 1.5 mm) were created in teeth by an expert dentist. To cut the teeth, the carbide milling No.271 (Jota Company), and a high-speed handpiece under cooling with water spray perpendicular to the longitudinal axis of the tooth were used. In addition, to increase the precision in the cavities, after performing each of the 5 cavities, the milling was replaced. Then using a ¼ round milling, some grooves were inserted in the cavities to increase the amalgam engagement. Afterwards, the teeth were restored with 1 unit amalgam capsules (High copper Admixed Alloy Non Gamma 2; ANA 2000, Nordiska Dental, Angelholm- Sweden) that were prepared in amalgamators according to the instructions of the manufacturer and were finally polished by burnisher. In the next stage, the restored teeth were transferred to fifty 5-ml cryotypes, and artificial saliva with the same volume of bristle above the tooth surface was injected to ensure that the surfaces of the teeth were completely covered. It should be noted that during all stages of the study, the cryotubes were sealed with the use of a Parafilm to prevent leakage of saliva and possible mercury vaporization away.

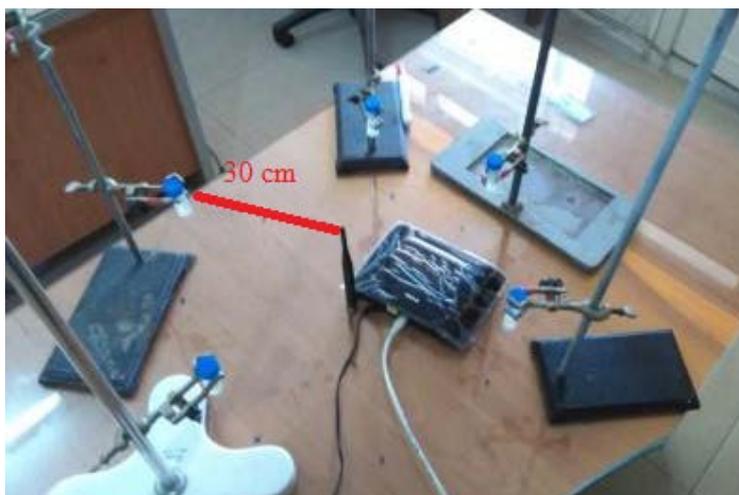


Figure 1. Wi-Fi router position and its proximity to the samples

Grouping of Samples Before Radiation

All 50 cylindrical cryotubes containing saliva and restored teeth that were prepared under the same conditions were classified into 5 randomized groups: the first group without radiation (control), the second group experienced radiation only by CT exam (CT), the third group samples were first subjected to CT exam and then Wi-Fi (CT+Wi-Fi), the fourth group first received Wi-Fi waves and then CT exam (Wi-Fi+CT), and the fifth group that received only Wi-Fi waves. In the following sections, each irradiation method is described in details.

Samples Exposure to Wi-Fi

All the groups with at least one radiation with Wi-Fi waves (Wi-Fi, Wi-Fi+CT, CT+Wi-Fi) were exposed to irradiation as follows. The cryotubes were placed on the perimeter of a circle with a radius of 30 cm by a clip in a way that the Wi-Fi device central antenna was placed at the center of the circle with the same distance from all the samples (30 cm from each sample), which is clearly depicted in **Figure 1**. Subsequently, the samples were irradiated by a D-Link Wi-Fi (wireless N150 home Router, China) through a wireless device connected to a laptop at a distance of 5 meters at the download mode with the same speed. The samples in the groups were continuously irradiated with wavelengths of 2.8 GHz by electromagnetic waves of the Wi-Fi device for 2 hours. It should be noted that the geometry of radiation in this study was based on previous studies [27].

Electromagnetic Field Calculation

To determine the Wi-Fi signals quantity, available power and electric field were measured. The relation between these parameters is based on pointing vector equation:

$$P = H \times E \quad (1)$$

It is presumed that the waves are transmitted in the atmosphere; hence, the relation between magnetic field (H) and electric field (E) components is as follows:

$$E = \sqrt{\frac{\mu_0}{\epsilon_0}} H = \sqrt{\frac{\mu_0}{\frac{1}{\mu_0 c^2}}} H = \mu_0 c H = 120\pi H \quad (2)$$

or

$$E = \sqrt{120\pi P} \quad (3)$$

In this equation, the received power can be calculated. The received power at the distance R from the source with power P_{in} and antenna gain G is calculated as follows [28, 29]:

$$P = \frac{P_{in} \times G}{4\pi R^2} \quad (4)$$

Table 1. The amount of released mercury (ppm) in subgroups (n = 5) with different radiation conditions after 24 and 48 hours. Also, p-values in the last row and the last column represent different levels of significance

| Time (h) | Values | Control | CT | CT+WiFi | WiFi+CT | WiFi | P-value (independent) Kruskal-Wallis Test |
|---|-----------|------------|------------|------------|------------|------------|--|
| 24h | Mean (SD) | 0.11(0.03) | 0.12(0.04) | 0.10(0.00) | 0.30(0.24) | 0.28(0.06) | 0.001* |
| | Min-Max | 0.16 | 0.1-0.18 | 0.1-0.1 | 0.11-0.73 | 0.2-0.33 | |
| 48h | Mean (SD) | 0.15(0.04) | 0.11(0.02) | 0.45(0.23) | 0.95(0.90) | 0.46(0.28) | 0.008* |
| | Min-Max | 0.1-0.2 | 0.1-0.13 | 0.2-0.78 | 0.1-2.00 | 0.14-0.86 | |
| P-value (pair) Wilcoxon Signed Ranks Test | | 0.066 | 0.285 | 0.043* | 0.345 | 0.138 | |

If $P_{in} = 1\text{Watts}$, $R = 30\text{cm}$, and $G = 1\text{dB} = 1.26$ then:

$$P = \frac{P_{in} \times G}{4\pi R^2} = \frac{1 \times 1.26}{4\pi \times (0.3)^2} = 1.11 \text{ Watt m}^{-2}$$

then:

$$E = \sqrt{120\pi P} = \sqrt{120 \times \pi \times 1} = 20.5 \text{ V m}^{-1}$$

Samples Exposure to X-ray

All the groups that had at least one X-ray radiation (CT, Wi-Fi+CT, CT+Wi-Fi) were irradiated as follows. The samples were transferred to CT-Scan center at Ali-Asghar Hospital affiliated to Shiraz University of Medical Sciences, located adjacent to the experimental environment. This unit is equipped with a GE Bright Speed 4-Slice CT-scan Machine (General Electric Healthcare Technologies, USA).

Samples were subjected to Para Nasal Sonius (PNS) CT-scans similar to a real patient. To do imaging from the coronal view, a patient is positioned in a prone position with neck extended and angulation perpendicular to the hard palate. Imaging was done from posterior margin of sphenoid sinus to anterior margin of frontal sinus. Some studies have indicated that the patient receives significant doses in the jaw and mandibular area [30- 31]. Other radiation factors stored after the end of the test were as follows:

Scan type: axial, Rotation time = 9, Rotation length full, no. images = 92, thickness: 2.5mm, intervals: 2.5, kV=120, mA=80, DLP=345.9 mGy-cm, CTDI vol =15 mGy.

Post-radiation Steps

After radiation exposure of the groups in the order described above, the samples in each group were divided into two subgroups. The first subgroup (consisted of 5 samples) was selected to investigate the release of mercury 24 hours after irradiation. To this end, after the mentioned time, the teeth were removed from the saliva and the cryotubes were again sealed to prevent saliva and mercury leakage. The second next 5 samples in each group were prepared in the same manner after 48 hours; samples were prepared to be sent to the laboratory for final analysis. All 50 samples were labeled and were transferred to a special laboratory for measuring heavy metals belonging to the Parham Gostar Fars Laboratory Complex equipped with the GBC932AA Mercury Vapor Spectrometry (GBC932AA), and GBC4501 Mercury Lamp.

To prepare the samples, they were first adapted with the temperature of the laboratory environment and placed in a shaker for 15 seconds to become more homogenous. One cc equivalent of about 1 g of each sample was weighed into a (porcelain) furnace, and then 3 cc HNO₃ and 1 cc HCl at high purity (MCC-Germany) were added under the hood to each sample. The samples were kept in the furnace for 24 hours at the room temperature, then were filtered with a funnel and a filter paper, and were prepared by a 10-centimeter balloon to be injected into a spectrophotometer. After reading the samples sent from the laboratory, the amount of mercury in each sample was measured in ppm and analyzed by SPSS software (Version 21). In addition, GraphPad software was used to plot the related graphs. Since, the data were not normal as it was indicated by Shapiro-Wilk test ($p < 0.05$), and given the low volume of data in each group, Kruskal-Wallis test and Wilcoxon Signed Ranks test were used to analyze the data, and non-parametric Post Hoc was performed for pairwise comparisons.

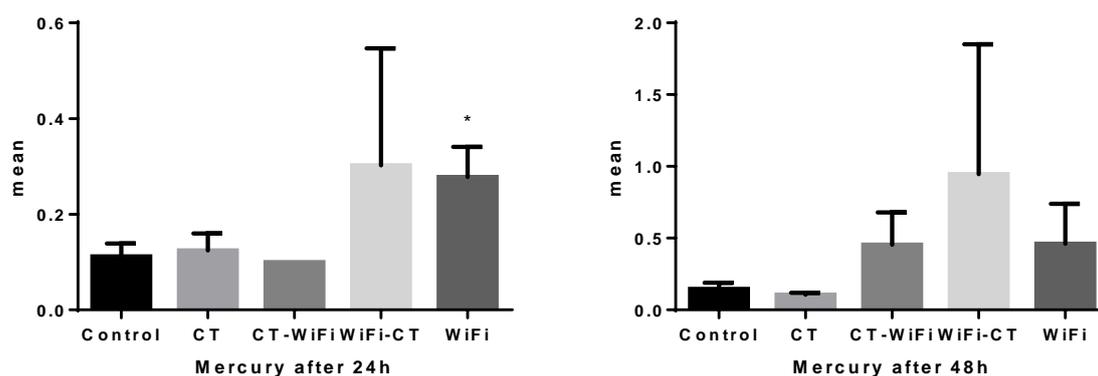


Figure 2. Mean values and standard deviations for mercury changes in subgroups based on the values presented in **Table 1**.

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics including mean, standard deviation, minimum and maximum scores for the release of mercury from amalgams in CT, CT-Wi-Fi, Wi-Fi-CT, and Wi-Fi subgroups as well as independent control groups after 24 and 48 hours. In **Figure 2**, the same mean values are also shown to describe the quality of these changes. As it can be seen in the graph, there are significant changes in the subgroups of 24 and 48 hours, as confirmed by p-values 0.001 and 0.008 in the last column of **Table 1**. In addition, the last rows of **Table 1** show pairwise comparisons for changes in the different subgroups over time. As it can be seen, only the released mercury in the CT+Wi-Fi subgroup shows a significant difference (p-value = 0.043). As the results of the pairwise comparisons for the radiated subgroups and the control group (no radiation) at 24 and 48 hours indicate, only the subgroup exposed to Wi-Fi waves showed a significant difference after 24 hours in comparison with the control groups (P = 0.033), while no significant difference was observed in the 48 hour group in comparison with the control group.

Here, it is necessary to raise a few issues on the release of mercury through its leakages from dental amalgams: First, its release has been proven clinically, and factors such as chewing, brushing, drinking hot liquids are believed to release mercury [32]. In addition, the electromagnetic waves interference is rapidly increasing, especially non-ionizing ones, which is a serious challenge in this regard.

As previous studies have pointed out, these waves can accelerate the release of mercury. The results of a study on 20 teeth in Class V cavities showed significance differences between two control and Wi-Fi router radiation groups (p = 0.009), pointing out the accelerated release of mercury as a result of irradiation [27]. This observation was in line with our findings. Moreover, in another in-vitro study, treated teeth and the effects of irradiation with MRI and X-ray fields were assessed separately (without exploring the combined effects), and was reported that X-rays could cause a significant difference (p < 0.05) in the release of mercury in comparison with MRI [25].

However, in our study the quality and manner of irradiation was closer to real conditions of dental irradiation in PNS CT-scan exam. We showed that X-rays alone could not make a significant difference in the release of mercury, which was in contrary to previous the study [25].

The second point considered here is the lack of sufficient information about the potential mechanism(s) underlying the release of mercury in the presence of electromagnetic waves that could be used to fully interpret and justify our empirical evidence. However, we barely managed to find references on the mechanism called "Triple M Effect" [33]. In this proposed mechanism mercury microleakage from amalgam-restored teeth was described in five steps. In short, in this mechanism, it is assumed that there is always a small gap between the amalgam and tooth made during the restoration process, called "marginal micro leakage", where saliva can fill this space. Hence, saliva exposed to these fields and the energy transfer followed by the subsequent temperature changes leads to the formation of "hot spots" and also fine gas bubbles that are rapidly expanded resulting in microleakage of mercury [33].

To validate the proposed mechanism, this difference was clearly observed in a group that was exposed to WiFi waves alone, but it was not the case with the other groups, where WiFi and CT waves (WiFi + CT, CT + WiFi) were combined. In other words, CT played the role of a protector by displacement of order, by doing so; different amount of mercury was observed. For instance, in the group that initially received Wi-Fi and then CT waves (Wi-Fi+CT), there was a significant difference over time in comparison with the CT+Wi-Fi group (p = 0.043). Therefore, it seems

that the proposed "Triple M Effect" mechanism and other subsequent mechanisms should pay more attention to the problem of different combination of electromagnetic waves (ionization and non- ionization).

To the best of our knowledge, this is the first study to have examined the combined effects of non-ionizing waves (Wi-Fi) and ionizing waves (CT) with different radiation patterns. A limitation in this study was the number of sample size. Consequently, nonparametric tests were used according to an expert opinion. Hence, we recommend further research in this field.

CONCLUSION

Despite the frequent usage of ionizing and non-ionizing waves in recent years, the effects of such waves on human health and the environment have become great concern for scholars in this filed. Especially the increasing emission of non-ionizing waves through communication devices such as cell phones, Wi-Fi routers, BTS antennas, etc... Additionally, various studies have shown the dangers of mercury toxicity in animal and human studies, and that is why the risk of mercury released from amalgams has become a challenging issue in the view of many scholars. In line with previous studies, our findings showed that these waves are not without any side effects on the release of mercury. Therefore, substituting amalgams in their present form seems to be required and serious reconsideration is essential.

ACKNOWLEDGMENTS

This article was extracted based on the results of a research project No.8920 that was financed by Research Grants from Shiraz University of Medical Sciences. The authors appreciate Shiraz University Research Deputy staff for their commitment to this project. We also extend our appreciation to Dr. Darafshi, the Research Deputy of Dentistry School of Shiraz University of Medical Sciences, for his support and permission to let us use the equipment of their laboratory. The authors also wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) at Shiraz university of Medical Sciences for his invaluable editorial assistance.

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