RESEARCH

The Relationship Between Plasma MicroRNAs and Serum Mercury Levels in Patients with Amalgam Filling and Dentists

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ABSTRACT

The Relationship Between Plasma MicroRNAs and Serum Mercury Levels in Patients with Amalgam Filling and Dentists

Background: The aim of this study was to investigate the relationship between serum mercury and some plasma microRNA (miRNA) levels associated with neurological diseases in patients with amalgam filling and dentists.

Methods: This study included 30 patients with amalgam filling, 30 dentists, and 30 healthy individuals as the control group. Circulating plasma miRNAs (124-3p, 125-5p, and 127-3p) were evaluated using real-time quantitative polymerase chain reaction analysis. The serum mercury levels were measured using inductively coupled plasma-mass spectrometry. ANOVA and Tukey's multiple comparison tests were used for statistical analyses ($\alpha = .05$).

Results: A significant difference in serum mercury and plasma miRNA levels was found between the groups. Significant positive correlations between serum mercury and plasma miRNA 125-5p and 127-3p levels were detected in the patient group (r: 0.56 and r: 0.39, respectively). Serum mercury and plasma miRNA-125-5p levels showed a positive correlation in the dentist group (r: 0.37).

Conclusion: Having amalgam filling caused a significant increase in serum mercury and some plasma miRNA levels (124-3p, 125-5p, and 127-3p). The presence of a positive correlation suggests that sensitivity to neurological diseases may increase because of mercury exposure. Alternative restorative materials should be preferred for amalgam filling and amalgam fillings should be replaced with other restorative materials under necessary preventions.

KEYWORDS

Dental amalgam, Mercury, MicroRNAs, Neurological disease, Restorative material

ÖZ

Amalgam Dolgulu Hastalarda ve Diş Hekimlerinde Plazma MikroRNA'lar ile Serum Civa Düzeyleri Arasındaki İlişki

Amaç: Bu çalışmanın amacı, amalgam dolgusu olan hastalarda ve diş hekimlerinde serum civa ve nörolojik hastalıklarla ilişkili bazı plazma mikroRNA (miRNA) seviyeleri arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Bu çalışmaya 30 amalgam dolgulu hasta, 30 amalgam dolgusu olmayan diş hekimi ve 30 sağlıklı kontrol grubu dahil edildi. Dolaşımdaki plazma miRNA'lar (124-3p, 125-5p ve 127-3p), gerçek zamanlı kantitatif polimeraz zincir reaksiyonu analizi kullanılarak değerlendirildi. Serum civa seviyeleri, endüktif olarak eşleşmiş plazma-kütle spektrometresi kullanılarak ölçüldü. İstatistiksel analizler için ANOVA ve Tukey'in çoklu karşılaştırma testleri kullanıldı ($\alpha = .05$).

Bulgular: Gruplar arasında serum civa ve plazma miRNA seviyelerinde anlamlı bir fark bulundu. Hasta grubunda serum civa ile plazma miRNA 125-5p ve 127-3p seviyeleri arasında anlamlı pozitif korelasyonlar tespit edildi (sırasıyla r: 0.56 ve r: 0.39). Serum civa ve plazma miRNA-125-5p seviyeleri, diş hekimi grubunda pozitif bir korelasyon gösterdi (r: 0.37).

Sonuç: Amalgam dolguya sahip olmak serum civa ve bazı plazma miRNA düzeylerinde önemli bir artışa neden olmuştur. Pozitif bir korelasyonun varlığı, civa maruziyeti nedeniyle nörolojik hastalıklara duyarlılığın artabileceğini göstermektedir. Amalgam dolgu için alternatif restoratif materyaller tercih edilmeli ve amalgam dolgular, gerekli önlemler alınarak diğer restoratif materyaller ile değiştirilmelidir.

ANAHTAR KELİMELER

Civa, Dental amalgam, MikroRNA, Nörolojik hastalık, Restoratif materyal

Dental amalgam has been used for restorative purposes for many years. Its advantages include its physical and mechanical properties, stability, easy application, and relative affordability compared with directly applied composite restorative materials.[1] One of the most serious concerns about dental amalgam is that it can be harmful to the human body and nature because it contains mercury.[1] Mercury vapor released when making and removing amalgam has been reported to show side effects on patients, dentists, and staff.[1,2] Whether these levels are safe enough remains unclear.[3] Neghab et al. reported that, the urinary concentration of mercury in dentists was significantly higher than the general practitioners. Additionally some symptoms such as muscular, neuropsychological, cardiovascular, respiratory and dermal were more prevalent in dentists.[4] It has been reported that; occupational mercury exposure in dentists is associated with the increase of prevalence of intoxication symptoms.[4] On the other hand; a meta analysis showed a low prevalence of neuropsychological deficits in occupationally mercury exposed persons compared with non-exposed workers.[5]

Some examples of the side effects of mercury in amalgam are associated with chronic fatigue, loss of

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strength, epilepsy, migraine, blindness, multiple sclerosis, and Alzheimer's disease (AD).[6-9] Blood mercury levels were found to be statistically higher in patients with Alzheimer's than in the control group. When associated with the number of amalgams, mercury level was higher in patients with one or more amalgams in the mouth than in those without, but this difference was not statistically significant.[10] Some researchers have suggested that an increase in mercury in the blood of a fetus or baby could be a potential cause of neurodevelopmental barriers.[2]

Individuals who have not been exposed to mercury vapor for a long time are expected to have whole blood mercury levels below 10 µg/L (50 nmol/L).[11-14] This value may increase when exposed to mercury vapor for a long time. As soon as exposure to mercury vapor ceases, mercury level in the blood and urine decreases, but the amount of mercury that accumulates in organs which those in the central nervous system (CNS), may still be high.[15]

Demonstrating the effects of microRNA (miRNA) in neurological diseases is not only helpful in understanding the etiology and pathophysiology of these diseases but also in developing more effective diagnosis and treatment methods. Abnormal miRNA functions have been reported to cause neurodegeneration.[16,17] One of the important miRNAs specific to the nervous system is miR-124.[18] The expression of miR-124 is enhanced in neurons, and its expression level rises over time in the advancing nervous system.[19-21] Furthermore, miR-125b-5p is part of a group of miRNAs that act as cahnges from the multiphase to neuron differentiation by inhibiting multiple and differential mRNA targets.[22] miR-127-3p has also been explained as a neuron-enriched miRNA playing a critical role in neuronal differentiation in the CNS.[20,23]

There is no restriction on the use of amalgam fillings in Turkey. Some information about amalgam filling obtained through television, newspaper, social media etc. causes patients to approach amalgam filling cautiously. However, the use of amalgam filling is still widely used in rural areas. To our knowledge, no study has yet evaluated the relationship between miRNA and amalgam filling in the literature. Thus, this study aimed to evaluate the relationship between the expression levels of some miRNAs, which are associated with neurological disorders (miR-124-3p, miR-125-5p, and miR-127-3p) and the serum mercury levels of patients with amalgam fillings and dentists who are occupationally exposed to elemental mercury.

MATERIALS AND METHODS

The study was carried out in accordance with the principles of Declaration of Helsinki.

Participants: This study was approved by the local ethics committee (date: May 15, 2019; no: 2019/211). Written and verbal consent was obtained from all the participants. The groups were as follows: 30 patients with at least one amalgam filling for at least five years in their mouths, 30 dentists who preferred to make amalgam filling and had no amalgam filling, and 30 healthy individuals without any amalgam filling as the control group. Patients and healthy participants consisted of individuals who came to the Necmettin Erbakan University Department of Restorative Dentistry for treatment. Characteristic data of all participants are presented in Table 1.

Table 1.

Demographic characteristics of participants

	Patients with amalgam filling (n=30)	Dentists (n=30)	Control (n=30)
Age (years)	33.9±5.6 min:23, max:44	31.6±5.4 min:25, max:43	34.3±6.2 min: 25, max: 44
Sex (male/female)	19 F, 11 M	22 F, 8 M	12 F, 18 M
Number of filling	2.6±1.1 min: 1, max: 7	-	-
Professional working years	-	8.5±5.02 min:2, max:21	-

F: Female, M: Male

The exclusion criteria of this study were systemic diseases such as: diabetes, hypertension, malignant diseases, chronic liver disease, cardiovascular disease, infectious diseases, pregnancy, alcohol and smoking habits, and using vitamin supplements, minerals, antioxidants, and fish oil tablets. The researchers examined the mercury content in the body in urine, blood, and hair samples.[24] Blood samples collected under the conditions of the Faculty of Dentistry were transferred to the laboratory in this study.[25] Blood samples were obtained after overnight fasting and placed into plain vacuum tubes and EDTA tubes. Plasma and serum samples were obtained after a suitable centrifugation and stored frozen at −80 °C until the day of study.

Measurement of miRNA expression levels: The miRNAs targeted from the participants' blood samples (miR-124-3p, miR-127-3p, and miR-125-5p) were studied. RNAs were isolated from the plasma samples using the RTA miRNA isolation kit (RTA Lab, Kocaeli, Turkey). The RNA samples were then converted to cDNA using the oneScript cDNA synthesis kit (ABM, Richmond, BC, Canada). The cDNA samples were pre-amplified using Poly (A) Polymerase Yeast (ABM,

Richmond, BC, Canada). The cDNA samples were pre-amplified using Poly (A) Polymerase Yeast (ABM, Richmond, BC, Canada). Quantitative real-time polymerase chain reaction analysis was performed using the BrightGreen miRNA qPCR MasterMix (ABM, Richmond, BC, Canada) on a Light Cycler 96 System (Roche Life Science, Mannheim, Germany). The relative gene expression was determined with the comparison of cycle times for the target PCR using the following formula: relative gene expression = $2^{-(\Delta Ctsample - \Delta Ctcontrol)}$.

Measurement of serum mercury levels: The serum mercury levels were calculated using inductively coupled plasma-mass spectrometry (Thermo Scientific ICAPQC, USA). All analyses of serum mercury levels were performed at once after all samples were taken and carried to the Yozgat Bozok University Science and Technology Application and Research Center (Occupational and Environmental Toxicology Laboratory) on dry ice. The parameters that used in this study were: radiofrequency power of 1550 W, nebulizer gas of 0.96 L/min, plasma gas of 0.88 L/min, nebulizer pressure of 3.01 bar, dwell time of 0.01 ms, and spray chamber temperature of 3.7 oC. The sampler probe was washed between injections by rinsing with ultrapure water for 30 s, followed by washing with 2% HNO_3 for 45 s and then rinsing with ultrapure water for 45 s. Each measurement was repeated three times for the average result. Serum sample of 0.25 mL was digested in teflon vessels with 5 mL suprapure HNO_3-5 mL deionized water in a microwave oven (Milestone D5, USA). The clear supernatant was transferred to polypropylene tubes and diluted to 20 mL with deionized water after cooling.

Statistical analyses: SPSS v. 21.0 (SPSS Inc., IL, USA) programme was used for statistical analyses. Groups of data were compared using ANOVA and Tukey's multiple comparison test. The correlations between serum mercury and the plasma miRNA variables were determined by Pearson's correlation test. All results are presented as the mean ± standard errors. Confidence interval was determined as $p < 0.05$.

RESULTS

No significant differences were observed in the serum mercury and plasma miR-124-3p, miR-125-5p, and miR-127-3p levels in the groups according to age and gender ($p > 0.05$).

The serum mercury and plasma miRNA levels of the groups are presented in Table 2.

Table 2.

Comparison of groups by serum mercury and plasma miRNA levels: ANOVA test results

	Patients with amalgam filling (n=30)	Dentists (n=30)	Control (n=30)	P*	P**	P***	Anova p level
Hg	5.84±0.13	5.33±0.08	5.25±0.09	0.001	0.872	0.004	0.003
miR 124-3p	9.13±1.44	0.99±0.20	0.92±0.10	<0.001	0.998	<0.001	<0.001
miR 125-5p	0.92±0.23	0.15±0.02	0.09±0.01	<0.001	0.936	<0.001	<0.001
miR 127-3p	8.87±1.35	1.84±0.43	1.39±0.22	<0.001	0.923	<0.001	<0.001

**p, Patients with amalgam filling group compared with control group.*
***p, Dentists group compared with control group.*
****p, Patients with amalgam filling group compared with dentist group.*
All values are mean ± standard errors.
miR: microRNA; Hg: Mercury

None of the subjects had mercury values above the upper limit. According to the ANOVA results, a statistically significant difference was found between the serum mercury levels and the levels of plasma miR-124-3p, miR-125-5p, and miR-127-3p ($p < 0.001$). The levels of serum mercury and plasma miR-124-3p, miR-125-5p, and miR-127-3p of the group of patients with amalgam filling were significantly higher than those of the dentist and control groups.

The results of Pearson's correlation test are shown in Table 3.

Table 3.

Pearson's correlation test results of serum mercury and plasma miRNA levels

	miRNA 124-3p	miRNA 125-5p	miRNA 127-3p
Patients with amalgam filling (n=30)	r: 0.264 (p: 0.159)	r: 0.563* (p: 0.001)	r:0.398*(p: 0.029)
Dentists (n=30)	r: 0.323 (p: 0.081)	r: 0.379* (p: 0.039)	r: 0.128 (p: 0.500)
Control (n=30)	r: -0.105 (p: 0.582)	r: -0.140 (p: 0.460)	r: 0.0844 (p: 0.657)

**Statistically significant (p<0.05)*

No correlation was found betsween the serum mercury level and the plasma miR-124-3p level in all groups. Significant positive associations were observed between the serum mercury level and the plasma miR-125-5p and miR-127-3p levels in the group of patients with amalgam filling (r: 0.56 and r: 0.39, respectively). Significant positive associations were found between the serum mercury level and the plasma miR-125-5p level in the dentist group (r: 0.37 and p: 0.03, respectively). No correlation was detected between the serum mercury level and the plasma miR-127-3p level in the control and dentist groups.

In the group of patients with amalgam filling, no relationship was found between the number of amalgam fillings and the levels of serum mercury and plasma miR-124-3p, miR125-5p, and miR127-3p (p: 0.263, p: 0.755, p: 0.859, and p: 0.733, respectively).

Professional working years were categorized into two groups, namely 10 years-below and over 10 years, and both groups had 15 dentists each. No relationship was found between professional working years and the levels of serum mercury, plasma miR-124-3p, miR-125-5p, and miR127-3p ($p > 0.05$, p: 0.257, p: 0.762, p: 0.197, and p: 0.764, respectively).

The frequency of making amalgam filling of dentists was also recorded and categorized into three groups: 3–5 per month, 3–5 per week, and more often. No relationship was found between the frequency of making amalgam filling and the levels of serum mercury, plasma miR-124-3p, miR125-5p, and miR127-3p ($p > 0.05$, p: 0.577, p: 0.694, p: 0.702, and p: 0.112, respectively).

DISCUSSION

This study determined whether serum mercury and plasma miRNA levels were associated with neurological degeneration in patients with amalgam fillings and dentists who make amalgam filling. Amalgam filling in the mouth significantly increased serum mercury and plasma miR-124-3p, miR-125-5p, and miR-127-3p levels in those participants.

Dental amalgam contains elemental mercury, which can evaporate at room temperature. All forms of mercury are toxic. Therefore, amalgam fillings carry a risk of both local and systemic side effects.26 Allergic reactions, oral lichen planus, gray-black discoloration in soft tissue, galvanic current due to contact with other metals have been stated that as local side effects of amalgam fillings.[27] CNS is considered an organ in which the effect of mercury vapor is important.[25] Mercury vapor may spread to the CNS, causing symptoms such as tremor, extreme irritability, forgetfulness, weakness, and visual disturbances. Kidney failure, peripheral neuropathy, and liver dysfunction may be observed later.[28] Dentists are exposed to elemental mercury both from the making of amalgam and from their own amalgam fillings.[29] Personal, office characteristics, and professional practice may also effect the level of mercury exposure.[30-33]

In the present study, no statistical difference was found in the serum mercury and plasma miRNA values between the dentist and the control group. Significant positive associations between serum mercury and plasma miR-125-5p levels were detected in the dentist group. Previous presence of amalgam fillings and other sources of mercury exposure in the control group may have affected the results. One limitation of this study is the limited number of dentists who participated. It considered that results may vary with more dentist participants who perform amalgam filling more frequently. A positive correlation between mercury and miR-125-5p suggests that it may be a risk for neurological diseases. Another limitation of the study is that the participating dentists did not evaluate the working conditions. Variables related to working conditions (e.g., surface conditions, size of the place, ventilation, and type of equipment) have been reported to effect the level of mercury.[34-36] Hock et al. reported higher blood mercury levels in Alzheimer's disease and major depression groups, regardless of the presence of amalgam fillings. They concluded that this may be due to other environmental exposure to mercury and should be investigated.[10] The last limitation can be stated that there is no question about possible mercury exposure sources.

Researchers have reported significant positive correlation between the number of amalgam fillings per day and the occurrence of neuropsychological and muscular disorders (e.g., memory deficit, hand tremor, and irritability) in dentists.[4] Dentists are exposed not only to mercury but also to many other chemicals (organic solutions, medical antiseptics, acrylate materials, etc.) throughout their professional life.[37] Therefore, it is inaccurate to think that the symptoms are caused only by exposure to mercury. Exposure to other chemicals has also been reported to have an effect.[4] Researchers have reported a positive relationship between higher blood mercury levels and elevated scores of psychoticism and anxiety (in SCL-90-R) and a negative correlation between logical memory (in the Wechsler Memory Scale-Revised test) and total retention score (in the Verbal Test of Memory Processes test) among dental personnel.[25] Some neurobehavioral symptoms, decreased psychomotor speed, decreased cognitive flexibility, attention deficit, memory loss, fatigue, and sleep disorders have been explained to be related with mercury content in amalgam.[15] Although no toxic cases have been reported, researchers report that dental amalgam may be associated with Alzheimer's Disease and Parkinson's disease with current data.[38]

Consistent with the literature, the serum mercury levels of patients with amalgam filling were higher than those of the other groups in the present study. This is considered to be the result of exposure to amalgam filling. In accordance with our findings, Pesch et al. found higher mercury levels in urine associated with the number of amalgam fillings and the number of amalgam filling surfaces.24 Unlike these researchers, Mc Grother et al. reported no positive relationship between body mercury content and the presence of amalgam filling and observed that the amount of filling was higher in patients with multiple sclerosis than in the controls.[39] Although

the relationship between dental amalgam and multiple sclerosis disease has not yet been proven, recent data suggest that repeated exposure to Hg vapor may increase the progression of MS through mitochondrial damage in recent experimental studies.[40] There was no relationship between the number of amalgam fillings and the levels of serum mercury and plasma miR-124-3p, miR125-5p, and miR127-3p in the present study. This may be related to the number of amalgam fillings that the participants have, the number of filling surfaces, the duration of the filling in the mouth, and the presence of teeth that contribute to chewing function. In addition, the presence of patients who previously had teeth with amalgam filling and were included in the study after extraction of these teeth may have been effective in these results. The number of amalgam fillings and surface area were found to be positively correlated with mercury in the blood, which was significantly lower in the group without amalgam fillings.[34] Additionally, general health complaints have been reported to decrease with the removal of amalgam fillings compared with the decrease in the previous urine mercury level.[26] No difference was found in the mean neurobehavioral evaluation or nerve conduction velocity in children treated with amalgam filling compared with those treated with composite resin.[41]

MiRNAs have an important role in basic biological processes, such as apoptosis, proliferation, differentiation, improvement, and inflammation.[42] One of the functions involving miRNAs is cell care, which is the processes involved in prenatal, postnatal, and adult CNS improvement.[43] MiRNAs have important contributions in CNS development and neurodegenerative processes.[44]

MiR-124-3p is one of the subspecies of miR-124.45 MiR-124 was downregulated in AD brain, and it was associated with the generation and accumulation of amyloid beta.[46,47] Researchers have reported that miR-124-3p could have a neuroprotective effect on AD by inhibiting the hyperphosphorylation of tau-induced cell apoptosis.[45] Similar studies have reported that miR-124-3p overexpression has a neuroprotective effect on vitro models of Parkinson's disease.[48] In the current study, the plasma miR-124-3p values were found to be higher in patients with amalgam filling than in the other groups. It has been shown that exposure to mercury through amalgam filling can cause a neurological effect.

One of the miRNAs that is upregulated during neurogenesis is miR-125b.[44] MiR-125b specifically promotes the generation of dopaminergic neurons.[49] The main sources of dopamine in the mammalian CNS are dopaminergic neurons in the midbrain. Their absence is associated with a neurological disease (i.e., Parkinson's disease).[50] Researchers have reported that miR-125b could support the continuation of differentiated neuronal cells by repressing apoptosis.[51] The absence of miRNA-125b encourages the storage of mitotic cells, a raise in cell death, and a reduce in differentiation, whereas the overexpression of miR-125b has the reverse effect.[51] Researchers have also reported that the overexpression of miR-125b in long-term, self-renewing neuroepithelial-like stem cells weakens their self-renewal and provokes differentiation into neurons.[52] In the present study, plasma miR-125-5p level was found to be higher in patients with amalgam filling than those in the other groups. Moreover, a positive correlation with serum mercury was detected in the group of patients with amalgam filling and the dentist group. Researchers have reported that changes in the expression of miR-125 isoforms can have a significant effect on cell destiny.[53]

These results can be associated with the contribution to the neurogenesis process against neurodegeneration. It can be an advanced protection mechanism against mercury exposure.

One of the possible biomarker of different neurodegenerative diseases is MiR-127-3p. It was found to be upregulated in AD serum and downregulated in AD cerebrospinal fluid compared with the neurologically normal age-matched controls.[54] Moreover, it was upregulated in progressive multiple sclerosis patients compared with the controls.55 As with other miRNAs, plasma miR-127-3p values were significantly increased in patients with amalgam filling. Plasma miR-127-3p also showed a positive correlation with serum mercury. The increase in values due to the mercury content in the amalgam filling showed a predisposition to miRNAs in neurological diseases in people with amalgam filling.

CONCLUSION

Within the limitations of the study, the following conclusions were drawn:

- The levels of serum mercury and the plasma miRNAs associated with neurological diseases (124-3p, 125-5p, and 127-3p) were found to be significantly increased in patients with amalgam filling.
- The positive correlation between the serum mercury level and the plasma miR-125-5p and 127-3p levels in patients with amalgam filling suggested that mercury exposure may be associated with neurological diseases.
- Long-term studies with more dentists are needed to clarify occupational mercury exposure in terms of neurological diseases.
- Alternative restorative materials should be preferred for amalgam filling and amalgam fillings should be replaced with biocompatible restorative materials under necessary preventions.

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