Trace elements and oxidative stress markers in saliva of subjects with amalgam fillings

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ABSTRACT

Background: Amalgam is the most frequently used restorative material for dental treatment. It is mainly used in posterior teeth, usually on occlusal surfaces as an economical, long lasting and durable filling material and represent the main source of exposure to mercury and other toxic metals (copper, tin, silver, etc.).

This study designed to measure oxidative stress marker malondialdehyde (MDA) and anti-oxidants (uric acid and glutathione) concentrations in saliva of subjects with amalgam fillings. And measure trace elements (copper, zinc) concentrations in saliva of subjects with amalgam fillings.

Subjects,materials and methods: Fifty subjects were participated in this study, they were between the age of (20-50) years with amalgam fillings (cases group) and fifty one subjects with no amalgam fillings (control group), they were gender and age matched to that of subjects with amalgam fillings. Informed consent and ethical approval was obtained. Each subject fill a case sheet questionnaire then examined by using sterile dental mirror and sterile dental probe to determine any oral manifestations and to calculate the number of amalgam filled teeth and the number of filled surfaces.

Results: The results obtained from this study showed that Oxidative stress marker (MDA) were increased while antioxidants (glutathione, uric acid) were decreased in saliva of subjects with amalgam fillings. Trace elements (copper, zinc) were higher in saliva of subjects with amalgam fillings; salivary copper was significantly higher in subjects with > 10 amalgam filled teeth. Salivary total glutathione was significantly correlated (negative correlation) with the number of filled teeth. Salivary copper was significantly correlated (positive correlation) with the number of filled teeth and filled surfaces.

Conclusion: This study revealed that amalgam fillings associated with increase in oxidative stress marker(MDA) and decrease in antioxidants (glutathione, uric acid).Trace elements (copper, zinc) increased in saliva of subjects with amalgam fillings.

Keywords: Oxidative stress, malondialdehyde , glutathione. (J Bagh Coll Dentistry 2012;24(3):63-66).

INTRODUCTION

Amalgam is the most frequently used restorative material for dental treatment**.** Concern has been raised about amalgam fillings because they contain elemental mercury, and very small amounts of mercury vapor are emitted from dental fillings, mercury is toxic because it induce production of free radicals and modifies the redox potential of the cells. Amalgam fillings are known to release significant amounts of mercury in saliva which could represent a continuous source of oxidative damage to oral tissues [1].

Dental amalgam fillings interacts in a complex way with the environment in the oral cavity as they are subjected to chemical, biological, mechanical and thermal forces. These forces change the restoration appearance and properties, while metal ions, amalgam debris, non metallic corrosion products and mercury vapor are released into the oral cavity [2].

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Amalgam corrosion is an oxidation-reduction reaction; the metals in the amalgam produce chemical compounds up on reaction with non metallic elements in the mouth.

Amalgam corrosion is important because it is one of the factors that determine how much mercury is released into the mouth from the fillings [3].

Oxidative stress represent imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to detoxify the reactive intermediates or to repair the resulting damage. The reactive oxygen species degrade unsaturated fatty acids and forming malondialdehyde. The production of this compound is used as a biomarker to measure the level of oxidative stress [4]. Glutathione is a protein composed of three amino acids: cystein, glutamic acid and glycine. Glutathione is important antioxidant counteracting the effects of free radicals produced in the body by oxidation reactions [5].

Uric acid is one of the most antioxidants in the body, it is heterocyclic compound of carbon, nitrogen, oxygen and hydrogen. It is created when the body breaks down purine during metabolism [6].

MATERIALS AND METHODS

Unstimulated whole saliva was collected from each individual, all subjects were healthy look without any signs and symptoms of any systemic diseases and were without gingivitis or periodentitis. Each subject asked to collect 5-10 ml saliva in the tube by spitting. After collection of saliva samples, it centrifuged at 3000 rpm for 15 minutes, the clear supernatant were separated and stored frozen at -20 ◦c until assayed.

Measurements of oxidative stress marker malondialdehyde (MDA):

Principle:

Lipid peroxidation end product ,particularly-malondialdehyde (MDA) react with thiobarbituric acid (TBA) under acidic condition and heating to give a pink color that measured spectrophotometricaly at 532 nm [7].

Measurement of salivary total glutathione (GSH) [8]:

Principle:

5,5 dithiobis 2-nitrobenzoic acid (DNTB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound.The absorbance of the reduced chromogen is measured at 405 nm and is directly proportional to the GSH concentration.

Salivary Uric acid measurements

Principle:

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide.In the presence of peroxidase (POD), a mixture of dichlorophenol-sulphonate (DCPS)and 4-aminoantipyrine(4-AA) is oxidized by hydrogen dye proportional to the concentration of uric acid in the sample [9].

Measurements of trace elements (Cu, Zn) in saliva:

The elements Cu and Zn under examination were determined using air-acetylene atomic absorption spectrophotometer (AAS). The principle of AAS measurement is as follow: the sample for analysis are dispersed in a beam of energy from a hallow-cathod lamp and atoms in the ground state absorb the incident energy of certain wave length. The absorption causes a decrease in emerging energy and with suitable instrumentation the decrease could be measured and the metal ions concentration was determined [10].

RESULTS

The results showed that the mean of salivary MDA in subjects with amalgam fillings was higher than the mean of salivary MDA in control subjects.

Statistical analysis using t-test showed that the mean of salivary MDA was significantly higher ($p<0.001$) in subjects with amalgam fillings than that in subjects without amalgam fillings.

The mean of salivary total glutathione, uric acid in subjects with amalgam fillings was lower than that in control subjects with statistically no significant differences.

The mean of salivary copper, zinc in subjects with amalgam fillings was higher than that of the control group with statistically high significant differences as shown in figure 1.

The results showed that no significant correlation has been found between salivary malondialdehyde concentration and the number of filled teeth while a significant negative linear correlation has been found between salivary total glutathione concentration and the number of filled teeth. No significant correlation has been found between salivary uric acid concentration and the number of filled teeth.

High significant positive linear correlation has been found between salivary copper and zinc concentrations and the number of filled teeth. No significant correlation has been found between salivary malondialdehyde , total glutathione, uric acid concentrations and the number of filled surfaces. Highly significant positive linear correlation has been found between salivary copper concentration and the number of filled surfaces while no significant correlation has been found between salivary zinc concentration and the number of filled surfaces.

The results showed that in the control subjects a significant negative linear correlation has been found between salivary MDA and salivary total glutathione using the correlation coefficient(r), while no significant correlation was found between salivary MDA and salivary uric acid .

In subjects with amalgam fillings no significant correlation has been found between salivary MDA and both salivary total glutathione and salivary uric acid as shown in table 1.

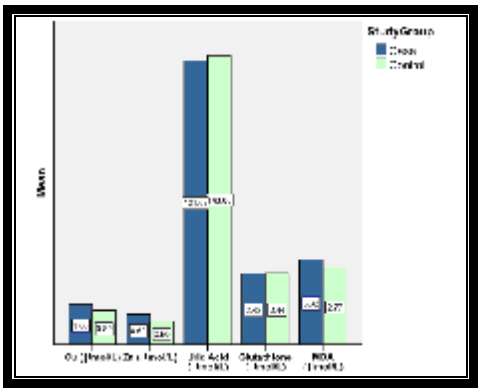

Figure 1: Mean levels of chemicals under study in both study groups.

Table 1: The correlation between salivary MDA concentration and both (total glutathione and uric acid) concentrations in each study group

	Cases (N = 50)		Control (N = 51)	
Chemicals (µmol/L)	**r**	**P**	**r**	**P**
Total glutathione	-0.138	0.340	*-0.299	0.033
Uric Acid	-0.066	0.651	-0.152	0.286

DISCUSSION

This study show that increase in oxidative stress marker (MDA) in saliva of subjects with amalgam fillings. Klinghardt and Mercola 2008[11]; Graham *et al*., 2009[12] found that the positive correlation of salivary malondialdehyde levels with the number of amalgam fillings and also with the number of filled surfaces could be attributed to that amalgam fillings represent a mixture of metals in an electrolyte(saliva),this result in galvanic current that pump mercury and other metals into the gums and oral mucosa from which it is carried throughout the body by the blood and nerves and the released mercury is toxic because it induce the production of free oxygen radicals and modifies the redox potential of the cells so represent a continuous source of oxidative damage to mouth tissues [13]. These results were in agree with the findings of [14] who found that there is a continuous release of mercury from amalgam fillings and there is a statically significant correlation between mercury concentration in saliva of subjects with amalgam fillings and the number of amalgam fillings and the release of mercury from amalgam fillings was increased with the increase in the number of filled surfaces. The results of this study also agree with the results of [15] who found that amalgam fillings bearers show significantly increased oxidative stress in saliva which correlates with the number of amalgam fillings. Lower concentration of salivary total glutathione in subjects with amalgam fillings than that of subjects without amalgam fillings have been found with statically no significant differences this due to that glutathione is the main natural chelator for heavy metals in the body due to it's sulfhydryl-containing cysteine, mainly mercury which is bound to glutathione to be capable to leave the body via urine or biliary excretion, thus high levels of glutathione is crucial for mercury metabolism.

Glutathione enzyme system act as a detoxifier by elimination of numerous toxins from the body including pollutants, heavy metals like mercury and lead, carcinogens, etc [16] so it's level decreased as a response to continuous release of mercury from amalgam fillings. At the same time glutathione act as antioxidant and it's level decreased as a result of oxidative Stress caused by mercury release from amalgam fillings [17].

These results agreed with [18] who found that very low mercury concentrations which are frequently seen in tissues of many people with dental amalgam leads to increased oxidative stress and reduction of glutathione concentrations which lead to sub cellular damage.

Although no significant differences was found in the mean of salivary uric acid between subjects with amalgam fillings and those without amalgam fillings but still it was higher in those with amalgam fillings.These results may be due to that uric acid is an important salivary antioxidants and it increased to counter act the increase in oxidative stress which is represented by saliva MDA, which was increased with the increase in the number of filled teeth, these result was agreed with Becker, 1993[19] also uric acid act as pro-oxidant according to the result of the study of Proctor, 1972[20]. The negative correlation between uric acid and the number of filled teeth may be due to that uric acid act as antioxidant against the oxidative stress that caused by mercury from amalgam fillings so uric acid may depleted to provide defense [21].

The result of this study showed that the mean of salivary copper was significantly higher in subjects with amalgam fillings than in saliva of subjects without amalgam fillings.These results may be due to electrochemical corrosion of dental amalgam which result in a conversion of the metallic solid components into dissolved metal ions and non metallic corrosion products.These results were agreed with [22, 23].

It has been shown that the mean of salivary zinc was significantly higher in subjects with amalgam fillings than that of subjects without amalgam fillings.

The positive correlation between salivary zinc and the number of filled teeth / surfaces may be due to electrochemical corrosion of dental amalgam which leads to release of components of amalgam fillings as metal ions and other corrosion products.Zinc thermodynamically is the most active components of dental amalgam and a fast preferential initial dissolution of zinc from zinc-containing amalgams had been reported [24, 25].

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