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Assessment of Oxidative Stress Induced by Various Restorative Materials: An *In Vivo* Biochemical Study

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***Abstract*:**

Background: To determine *in vivo* oxidative stresses induced by dental amalgam, composite resin, and glass ionomer cement (GIC).

Materials and Methods: A total of 60 patients were selected for the study between the ages of 15 and 40 years. Informed consent and Institutional Ethical Approval was obtained. The samples were divided into three groups depending on the type of restorative material planned for them, i.e., silver amalgam, composite resin, and GIC. Saliva was collected before restoration, 24 h, 7 days, and 14 days after restoration. The salivary oxidative stress malondialdehyde (MDA) was measured and statistically analyzed using Mann–Whitney test, Kruskal–Wallis test, and Friedman's test.

Results: The results show that the patients treated with amalgam restoration had shown significantly higher values as compared to composite and GIC at all the three-time intervals studied.

Conclusion: This study revealed that amalgam fillings were associated with the highest oxidative stress marker MDA as compared with composite and glass ionomer restoration at all the three-time intervals studied. Even after 14 days, the values were much higher indicating free ion leaching from the restoration.

Key Words: Malondialdehyde, oxidative stress, saliva

Introduction

Today, a wide array of alloys is used in dentistry, especially in restorative dentistry. So, biocompatibility and toxicity of these alloys have been a matter of concern while selecting restorative materials.[1] Restorative materials such as composite resin, adhesives, glass ionomer cement (GIC), and silver amalgam restoration are most commonly used.

These materials are exposed to factors such as temperature, pH, mechanical stress (corrosion), and microflora, which may lead to release of ions from these restorative materials. However, leaching of substances from the restorative materials is an important factor when biocompatibility of material is assessed.[2] These dental restorative materials have long-lasting contact with the oral environment and thus may affect the composition and properties of saliva.

Dental amalgam is the most common restorative material and has been widely used for over centuries. The most controversial side effect of amalgam, when used as a dental restorative material, is mercury leakage. Some researchers believe that it is minimal, much less than daily mercury intake with water, food, and air, while others are convinced that temperature changes, acids from dental plaque, salivary proteins, enzymes, brushing, and chewing cause its significant increase. Higher mercury levels were observed in the saliva, blood, urine, and feces of patients treated with amalgam restorations. Dental amalgam can also cause discoloration due to higher mercury concentration in restored and neighboring teeth. It can also affect oral mucosa and induces changes such as amalgam tattoo, lichen planus, and leukoplakia.[3]

Composite resin materials have an influence on dentin and dental pulp it causes chemical irritation which elucidates various degrees of inflammatory pulp response.[3] Polymerization shrinkage and bacterial microleakage play most important role in inflammatory pulp reaction in teeth restored with composite materials. The release of resin factors such as Bisphenol glycol metha acrylate, triethylene glycol dimethacrylate, 2-hydroxyethylmethacrylate, and urethane dimethacrylate under the influence of temperature changes, ethanol, enzymes, acids, and water has been proved. These elements have cytotoxic, genotoxic, and mutagenic effects on epithelial cells, fibroblasts, and monocytes. Disturbance in hormonal balance due to conversion of Bis-GMA into Bisfenol-A also associated with use of composite resin materials.[3]

Luting materials such as GICs, which were first introduced in the early 70s by Wilson and Kent. Since their introduction, glass ionomers have increasingly been used for restoration, cementation, cavity lining, bases, and core build-up. Major changes have occurred in their chemical composition[4] such as leakage of fluoride occurred during the 1st week, with the most rapid release during the first 24 h. During the 2nd week, the fluoride release was substantially lower and thereafter gradually and slowly leveled off during a 12-week period. The leaching of fluoride ions has been reported as an advantage of conventional GICs and supporting remineralization of adjacent enamel and prevention of secondary caries.[4]

The mouth is always moist due to the presence of saliva and is continuously subjected to fluctuations in temperature and a wide range of pH of saliva in the oral cavity contains various.

Biologically active substances such as enzymes, proteins and hormones, epithelial cells, numerous blood formed elements, and bacteria. All of these environmental factors contribute to the degrading process of dental restorative materials.[3] Free radicals get released from these restorative materials which affect oral health.

In 1994, Riley defined free radicals as reactive chemical species having a single unpaired electron in an outer orbit. This unstable configuration creates energy which is released through reactions with adjacent molecules such as proteins, lipids, carbohydrates, and nucleic acids. Most of the free radicals damaging the biological systems are oxygen-free radicals generally known as "reactive oxygen species."[5] The generation of free radicals leads to oxidative stress.

Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It interferes with the lipid peroxidation cycle and also causes oxidative DNA damage. It also causes physiologic adaptation phenomena and regulation of intracellular signal transduction.[6]

It interferes with the lipid peroxidation cycle and also causes oxidative DNA damage. It also causes physiologic adaptation phenomena and regulation of intracellular signal transduction. Reactive free radicals can produce chemical modifications and damage carbohydrates, proteins, lipids, and nucleotides in the tissues.[7] Reactive free radicals are able to damage cells by the start of lipid peroxidation causing a major change in the structural integrity and functions of cell membranes. When these free radicals react with lipids, the reaction is lipid peroxidation forming a by-product malondialdehyde (MDA).

The levels of MDA are used as a biomarker for the measurement of oxidative stress *in vivo*.

Materials and Methods

Method

About 60 healthy patients within the age group of 15-40 years, reporting to the Department of Conservative Dentistry and Endodontics, CSMSS Dental College and Hospital, were selected for the study. Written consent was concurred from all the patients. Patients already having multiple restorations were excluded from the study. The patients were randomly divided into three groups (n = 20).

1. Group A - Consists of 20 patients treated with silver amalgam restorations.
2. Group B - Consist of 20 patients treated with composite resin restorations.
3. Group C - Consist of 20 patients treated with GICs restorations.

For each patient, Class I cavity was prepared and filled with appropriate restorative material.

Sample preparation

About 5 ml of unstimulated whole saliva was collected and stored from each participant at four different occasions, i.e. before restoration, 24 h, 7 days, and 14 days after restoration.

The saliva was centrifuged at 3000 rpm for 15 min in centrifuging machine (REMI), the clear supernatant were separated from it and stored frozen at −20°C until assayed.

Measurements of oxidative stress marker

MDA measuring procedure

To 0.5 ml each of saliva and MDA standards, 2.5 ml of 20% trichloroacetic acid and 1 ml of thiobarbituric acid were added and mixed well. Then, the mixture was boiled in hot water bath for 30 min. After cooling in cold water, the resultant chromogen was extracted, with 4 ml of n-butyl alcohol and separation of organic phase was done and then centrifuged at 3000 rpm for 10 min. Absorbance rate of the butyl alcohol extracts of standards as well as samples were measured at 530 nm against distilled water as blank. The standard curve was plotted, and the concentration of total MDA in samples was calculated and expressed as MDA in nmoles/ml from the curve.

Results

The results showed that the mean of salivary in subjects with amalgam fillings was higher than that of composite and GIC.

Statistically analysis using Friedman's test was used to observe the change in the median MDA levels along the time trend.

Median MDA levels increased significantly over a period in Group 1 ($P < 0.001$) and Group 2 ($P = 0.001$) (Table 1 and Graph 1).

In Group 3, though median MDA levels increased on the 14th day as compared to baseline pre-treatment levels, the difference was not significant ($P = 0.37$). Kruskal–Wallis test was used to observe the difference in the median MDA levels among the three groups. Median pre-treatment and 24 h MDA levels did not differ significantly among the three groups (P = 0.81 and 0.44, respectively) (Tables 2-4).

Table 1: Group 1 (amalgam restoration).

Saliva collection	*N*	Median	SD	Percentiles			Mean rank	*P* value
				25th	50th	75th		
Pre-treatment	20	1.108	0.53250	0.6375	1.200	1.5000		
24 h	20	1.4250	0.71079	1.2000	1.3500	1.5000	1.83	0.000
7 days	20	2.1000	0.68889	1.5000	2.1000	2.7000	3.00	
14 days	20	2.7250	0.52245	2.4000	2.7000	3.0000	3.83	

SD: Standard deviation

Table 2: Group 2 (composite resin restoration).

Saliva collection	*N*	Mean	SD	Percentiles			Mean rank	*P* value
				25th	50th	75th		
Pre-treatment	20	1.3792	0.81616	0.7500	1.3500	2.1000		
At 24 h	20	1.4917	0.72702	0.9750	1.3500	2.1000	2.29	0.001
At 7 days	20	1.8667	0.75719	1.2000	1.5000	2.6750	3.67	
At 14 days	20	1.4083	0.64415	0.9000	1.2000	1.8000	2.17	

SD: Standard deviation

Table 3: Group 3 (glass ionomer restoration).

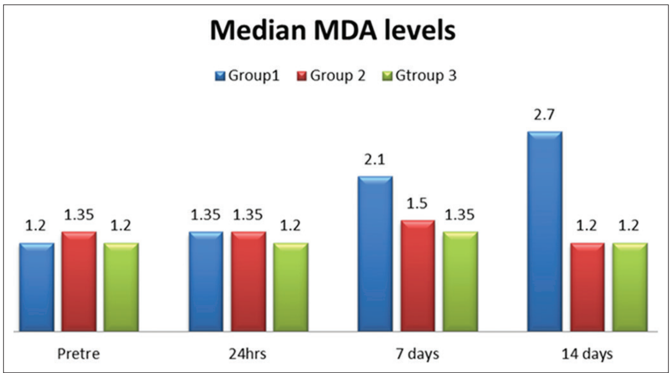
Saliva collection	*N*	Mean	SD	Percentiles			Mean rank	*P* value
				25th	50th	75th		
Pre-treatment	20	1.2625	0.47248	0.9000	1.2000	1.5000		
At 24 h	20	1.0792	0.53660	0.7875	1.2000	1.5000	2.04	0.366
At 7 days	20	1.3500	0.55432	0.7500	1.3500	1.7250	2.67	
At 14 days	20	1.3958	0.44947	1.2000	1.2000	1.7250	2.88	

SD: Standard deviation

Table 4: Kruskal–Wallis test.

Saliva collection	Group 1			Group 2			Group 3		
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median
Pre-treatment	1.1083	0.53250	1.2000	1.3792	0.81616	1.3500	1.2625	0.47248	1.2000
24 h	1.4250	0.71079	1.3500	1.4917	0.72702	1.3500	1.0792	0.53660	1.2000
7 days	2.1000	0.68887	2.1000	1.8667	0.75719	1.5000	1.3500	0.55432	1.3500
14 days	2.7250	0.52245	2.7000	1.4083	0.64415	1.2000	1.3958	0.44947	1.2000

SD: Standard deviation



Graph 1: Median malondialdehyde levels.

Whereas median MDA levels on 7 days and 14 days differ significantly across the three groups (P = 0.04 and <0.001, respectively (Table 5).

To see whether the median MDA levels differ among the groups, Mann–Whitney U-test was applied as part of the *post-hoc* analysis (Table 6). Median MDA levels changed significantly on the 14th day when the comparison was done among Group 1 and 2 as well as in 1 and 3. Significance was also shown on the 7th day among Group 1 and 3.

Discussion

This study shows that increase in oxidative stress marker MDA in the saliva of subjects with amalgam fillings as compared with composite and GIC. Dental Amalgam restorations are known to release significant levels of mercury in the saliva which could represent a continuous source of oxidative damage to the tissues. Mercury is the major component of tooth filling material and has been used for years because it is stable, economical, and easy to manipulate. The mercury vapor released from dental amalgam is inhaled, absorbed by the lungs, gastrointestinal tract, and jaw tissues, retained mostly in kidney, liver, central nervous system determined from the human autopsy. The release of mercury in the oral cavity may be due to the effect of biological corrosion due to bacteria, chewing, brushing, variations in pH of saliva, and oral temperature.[8]

In recent years, resin-based restorative materials use has increased in dentistry for the purpose of better esthetics,

Table 5: Chi square test

Chi square test	df	Chi-square value	*P* value
Pre-treatment	2	0.418	0.811
24 h	2	1.626	0.444
7 days	2	6.565	0.038
14 days	2	18.758	0.000

Table 6: *Post-hoc* analysis.

Time	Groups		
	Group 1 and 2	Group 2 and 3	Group 1 and 3
Pre-treatment	0.539	0.953	0.618
24 h	0.976	0.275	0.270
7 days	0.328	0.112	0.012
14 days	0.000	0.768	0.000

improved adhesion to enamel and dentine, and worries about adverse effects of mercury from amalgam. It has been shown that various components of dental composite resins can be released from restoration. These unpolymerized monomers can be leached into saliva and can cause adverse reactions. Resin-based dental materials such as composite resins come into direct contact with oral mucosa and can result in adverse reactions on oral mucosa.[9]

Previous studies had identified that some components of adhesives, restorative composite resins, and GICs are toxic. The mechanisms of cytotoxicity are related mostly to the small release of free monomers occurring during the monomer–polymer conversion. Second, long-term release of leachable substances is generated by erosion and degradation over time.[10]

Saliva modulates the ecosystem in the oral cavity and plays a critical role in oral homeostasis. In addition, saliva is the first line of defense against free radical-mediated stress. Salivary antioxidant defense mechanisms seem to be very important.[11]

Unstable configuration of free radicals creates energy which is released through reactions with adjacent molecules such as proteins, lipids, carbohydrates, and nucleic acids. Most of the free radicals damaging biological systems are oxygen-free radicals.[5]

In our study, we had selected 60 patients from the outpatient department of conservative dentistry and endodontic. The patients were healthy and not having any periodontal or gingival problems.

These 60 patients were divided into three groups of 20 patients each. Group 1 was amalgam, Group 2 was composite, and Group 3 was GIC restorations. Saliva sample was collected from each patient in morning hours. The sample was collected before treatment, 24 h after treatment, 7 days after treatment, and 14 days after treatment. Oxidative stress marker such MDA were also checked. The obtained data were analyzed statistically.

Robert, in 1990,[12] conducted a study on the health effects after dental amalgam removal. He suggested that the mercury vapors released from dental amalgam are directly related with the number of fillings done. Mercury in vapor is the elemental form. An average of 75% 6 to 80% 7 can be absorbed through alveoli when inhaled, and it then enters the blood stream. The elemental mercury passes through the blood brain barrier before it is ionized and get retained in the brain. Mercury accumulates in tissue after release from dental amalgam.

The results of composite restoration for MDA is in accordance with the study done by Pinar *et al.*, 2014.[13] The study show that the quantity of monomers leached into saliva reported a statistically significant increase during 7 days, and the major amount of release was seen generally in the 7 days, and the release in monomers except for Bis-GMA showed a significant decrease in 30 days ($P < 0.05$).

The amount of MDA molecule that is commonly used in oxidative stress measurements was investigated in this study. Our study shows that there is a statistical difference observed between 7th and 14th day, and in between 24 h and 7th day. However, there was no difference between pre-treatment levels and 7th day and in between pre-treatment levels and 14th day.

The study conducted by Gupta *et al.*, in 2012,[14] was also in accordance with our study. The author had done a study on release and toxicity of dental resin composite. Dental resin composite restorations are used as a posterior restorative material in traumatized and destructed tooth as an alternative to amalgam.

In our study, the stress released from glass ionomer restoration shows no significant difference. However, we observed that highly significant difference was between 24 h and 7th day after treatment, but there was no significant difference among rest of the days comparison. If we compare Group 1 with Group 2 and Group 2 with Group 3, Group 1 with Group 3, we observed that all the three groups show no significant difference.

The results were in accordance with the study done by Neelakantan *et al.*, 2011.[15] The author studied about the fluoride release which was measured every 24 h for the first 7 days and on days 14, 21, and 28, a combination fluoride ion selective electrode connected to an ion analyzer.

Despite the desirable property of fluoride release, conventional glass ionomers have several disadvantages such as water sensitivity, poor strength, and low occlusal wear resistance. The high sensitivity of these cements to water causes erosion of the glass ionomers during the early setting period, and this further increases the elution of fluoride. The quantitative leaching of fluoride is increased by the presence of cracks and pores on the surface of the material, leaving behind a low amount of residual fluoride.

Thus, this study emphasizes that the oxidative stress release from amalgam is comparative higher with composite and GIC. The merit of this study is that the study is non-invasive, easily available with more reliable results. Furthermore, it contributes to research to assess the importance of stress released by various restorative materials.

Saliva is used as a diagnostic tool in this study, which is easily available but frequently underestimated. The detection of certain substances in the saliva can be a marker of pathological changes not only in the mouth but also in the whole body. However, further studies are required by including more number of patients with multiple carious lesions, and more follow-up period.

Despite such valuable results, this study also carries some limitations which include the sample size, subjective assessment, and more technique sensitive. Considering the merits and demerits of this study, it can be suggested that the study is continued on a large sample of the population considering all the variables so that we can have a more definite result on a large scale basis.

Until now, no study had been done to compare the oxidative stress released from three commonly used restorative materials with each other. So, many more studies are further required to know the stress levels in the patients treated with dental restorative materials with extended observation period and increased sample size with multiple restorations.

Conclusion

Saliva is used as a diagnostic tool in this study, which is easily available but frequently underestimated. The detection of certain substances in the saliva can be a marker of pathological changes, not only in the mouth but also in the whole body. Finally, conclusion of this study states that if the level of oxidative stresses increases which leads to certain amount of harmfull effects on the patients body.

However, further studies are required by including more number of patients with multiple carious lesions, more follow up period.

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