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**Research Article**

# SALIVARY LEAD LEVEL AS A BIOMARKER FOR DENTAL CARIES –A CROSS SECTIONAL CLINICAL STUDY

**Shantanu Choudhari[1] and Girish Parmar[2]**

[1]Department of Pedodontia, Government Dental College and Hospital Ahmedabad, Civil Hospital campus, Asarwa, Ahmedabad-380016, Gujarat, India
[2]Government Dental College and Hospital Ahmedabad, Civil Hospital campus, Asarwa, Ahmedabad-380016, Gujarat, India

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**ABSTRACT**

**Purpose of the study:** The purpose of the study was to examine the association between salivary lead level and dental caries in children of age group 3-6 yrs.
**Materials and method:** It was a cross sectional study with sample size of 100 children in both control group (dmft=0) and study group (dmft>5)**.** Samples of unstimulated saliva were collected from all subjects and lead estimation was done by ICP-MS method. Unpaired student-t test was used for statistical analysis, with the level of significance set at 0.05.
**Results:** A highly significant correlation was found between salivary lead level and the presence of dental caries. The average values of lead in the control and study group were 2.84 mcg/l and 8.0 mcg/l, respectively.
**Conclusion:** The salivary lead levels were found to be higher in subjects with dental caries proving the cariogenic potential of lead.



## INTRODUCTION

Metals and their compounds found in all living organisms play many significant roles. Nevertheless, when their exposure is excessive they may cause toxic effects. Role of trace elements in the development of tooth decay has been an area of interest since the identification of the protective effects of fluoride [1]. Dental epidemiology provides some of the most convincing evidence that trace elements can affect the health of communities, owing to the variations in the regional distribution of caries [2]. The etiology of dental caries may be attributed in part at least to exposure to trace elements such as Selenium, Vanadium, Molybdenum, Strontium and Lead. Out of all these trace elements, lead remains a significant pollutant. It has acute cyanogenic and chronic effects on many tissues and accumulates in hard tissues of the body [3]. There are many animal and human studies which support the concept that lead is a caries promoting element [4]. The evaluation of metal content in biological fluids and tissues (e.g. blood, urine, saliva and teeth) can provide information about the level of intoxication and possible adverse health effects.[5,6]

Lead is a relatively heavy metal with atomic number 82 and has an atomic mass of 207.2 [7]. The word Lead was coined by Axon. It is chemically symbolized as Pb and comes from the Latin word Plumbum. It is a widely used metal such as for application in metal products, cables, pipelines, paints and pesticides. But at the same time it is a subtle and persistent `poison, with no biological or nutritional value. Lead is non-essential for living organism.

Human activities and extensive use of lead in various fields have resulted in its redistribution in the environment leading to contamination of air, water, and food [8]. Significant exposure to lead is an environmental threat to optimal health and to physical development in young children that affects all so-cioeconomic groups[9]. Toddlers and infants in the neonatal period are at high risk because of their hand to mouth practice[10]. Lead enters our body through two different routes: inhalation and ingestion [11]. Sources of lead are paints, painted toys, folk medicines, ayurvedic medicines, gasoline additives, cosmetics, lead glazed ceramics, dust, and potteries [12]. Besides the settling of atmospheric lead, surface contamination also occurs from contact with industrial waste containing lead [13].

**Corresponding author:* **Shantanu Choudhari**
Department of Pedodontia, Government Dental College and Hospital Ahmedabad, Civil Hospital campus, Asarwa, Ahmedabad-380016, Gujarat, India

In dentistry, lead is found in metal brackets, orthodontic appliances and intraoral x-ray films.[14] In addition, if dental assistants do not wash their hands or change their gloves after processing intraoral films, lead oxide might adhere to the gloves or hands and be introduced onto instruments and equipments entitled for use in the mouths of patients. This is important because inorganic lead is easily dissolved in human saliva [15].

Plasma, serum, saliva, bone, hair, nail, urine and feces can be used as a biomarker of lead instead of blood . Biomonitoring for human exposure to Pb reflects an individual's current body burden, which is a function of recent and/or past exposure. Thus, the appropriate selection and measurement of biomarkers of Pb exposure is of critical importance for health care management purposes, public health decision making, and primary prevention activities [17].

There is scarcity of data regarding the association of salivary lead levels and dental caries in the Indian literature. Hence this study was carried out to explore the possible association between idiomatic salivary lead level and dental caries.

## MATERIALS AND METHOD

This study was carried out in the Department of Pedodontics and Preventive Dentistry of Government Dental College and Hospital, Ahmedabad and included children of age group 3-6 years. The study was approved by the Institutional Ethical Committee. The study sample was equally divided into two groups of 100 each: Group A consisting of 100 children of control group with dmft score '0' and Group B consisting of 100 children of study group with dmft score more than 5. In both the groups, unstimulated whole salivary sample was collected. Informed consent was obtained from parents/guardians of all children included in this study.

In this study, children of 3-6 years of age and their parents were included. Children who were willing to give their salivary sample were included. Parents who were willing to sign the consent were included. Children without any metal appliances or silver amalgam filling and without any systemic disease or blood disorders were included. The subjects included had the habit of tooth brushing twice a day and with a diet history showing no in between snacking and lesser intake of sweet foods. The subjects who did not meet these criteria were excluded from the study. The subjects who did not meet these criteria were excluded from the study.

Each child received a thorough dental clinical examination for dental caries by two qualified dental personnels, according to a strict, well tested protocol (WHO criteria for assessment of dental caries**).** For this purpose, kappa analysis was performed and both examiners were in agreement with each other. Teeth were cleaned if necessary. Every surface of tooth to be examined was dried and later examined with mouth mirror and probe. All decayed, missing and filled teeth were recorded and scores allotted as per dmft index.

***Method of salivary sample collection:*** Unstimulated whole saliva sample were collected under the same condition by qualified dental personnels. Subjects were refrained from eating, drinking and oral hygiene for at least 2 hours before saliva collection to decrease probability of contamination of sample.5 ml of non-stimulated whole saliva was collected into 15 ml lead free plastic container. All the saliva collections were carried out between 8 am and 11 am to prevent circadian bias.

***Laboratory method***

Lead estimation done by ICP-MS (Make: Agilent 7700 MS [2013]) was used in NABL (National Accreditation Board for Testing and Calibration Laboratories) certified laboratory by well trained staff. ICP- MS stands for Inductively Coupled Plasma-Mass Spectrometry. Lead estimation by this process is divided in 2 parts: 1. Digestion procedure of saliva sample 2. Lead estimation in saliva sample by ICP-MS

***Digestion procedure***

All glassware and plastic ware were immersed in nitric acid overnight and rinsed with ultra purified water to eliminate lead contamination. The saliva sample was prepared for analysis of lead. The 2 ml saliva sample was digested using 3 ml of 50% nitric acid and the sample was diluted to 5 ml ultra purified water with the help of micropipette. After preparing all the samples, all tubes were placed in microwave digestion machine (Make: CEM Model: Mars Express, 2012) which used 50 degree to 220 degree ramping for 2 hours. In the digestion procedure, all organic portion is disintegrated and inorganic portion makes salt with nitrates. So at the end of digestion procedure lead is converted into lead nitrate. Samples were kept isolated to attain the room temperature, after which the amount of lead was detected using ICP-MS.

***Lead estimation by ICP-MS***

Elements are digested in a nitric–hydro chloric acid solution. Analytes in solution are introduced by pneumatic nebulization into radiofrequency plasma where energy transfer processes cause desolvation, atomization, and ionization. Ions are extracted from plasma through differentially pumped vacuum interface and are separated on the basis of mass-to-charge ratio by a quadrupole mass spectrometer having minimum resolution capacity of 1 atomic mass unit (amu) peak width at 5% peak height.

Ions transmitted through quadrupole are detected by continuous dynode electron multiplier assembly, and ion formation is processed by data handling system. After introduction of reference samples, all salivary samples are introduced and lead concentration values are recorded in computerized system.

***Statistical analysis***

Unpaired student-t test was used for statistical analysis, with the level of significance set at 0.05.

## RESULTS

The present study was a cross sectional study carried out by simple sampling method and the data was quantitative. Kappa analysis was performed for the two examiners who carried out the diagnostic examination of the children and both examiners were in agreement with each other. Kappa value was 0.79 and 0.86, respectively for control and study group. All data were encoded and compiled into a computer database. The data obtained was statistically analyzed using Independent student t-test (p- value $<0.05$ was considered significant).A highly significant correlation was found between salivary lead level and the presence of dental caries with p value $< 0.001$ (Table 3,figure 3). Subjects with dmft 0 had a mean salivary lead level

of 2.84 mcg/l while those with dmft > 5 had a mean salivary lead level of 8.00 mcg/l (Table 1,2, Figure 1,2,).

**Table 1** Salivary lead levels found in control group

| Salivary Lead Levels | | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| dmft | 0 | 100 | 2.84 | 2.02 | .202 |

**Table 2** Salivary lead levels found in study group

| Salivary Lead Levels | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| dmft >5 | 100 | 8.00 | 3.92 | .392 |

**Table 3** Salivary lead levels found in both the groups

| dmft | | N | Mean | Std. Deviation | Std. Error Mean | Mean Difference | P Value |
|---|---|---|---|---|---|---|---|
| Salivary Lead Levels | 0 | 100 | 2.84 | 2.02 | .202 | -5.16 | <0.001 |
| | > 5 | 100 | 8.00 | 3.92 | .392 | | |

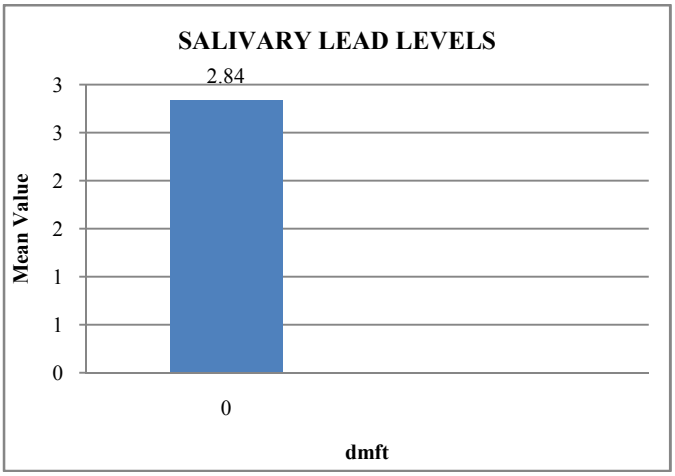


**Figure 1** Salivary lead levels in the control group

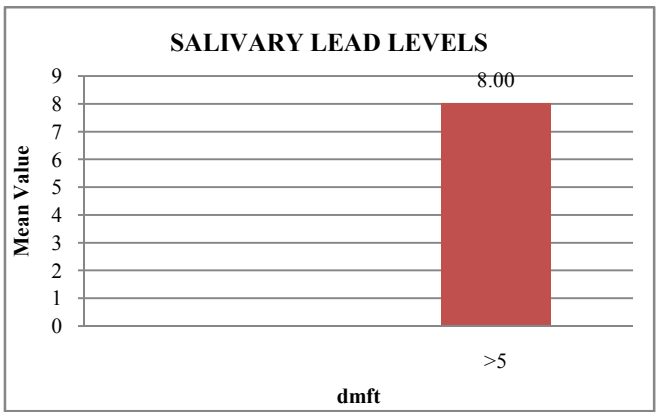


**Figure 2** Salivary lead levels in the study group

## DISCUSSION

Extensive use of lead for industrial purposes along with other human activities have resulted in its redistribution leading to contamination of air, water, and food which in turn results in significant rise in lead concentration in human body, especially in a developing country like India.[18] The severity of lead toxicity depends on the duration, frequency, and amount of exposure. Cumulative absorption of small amounts of lead in the body usually results in lead poisoning.

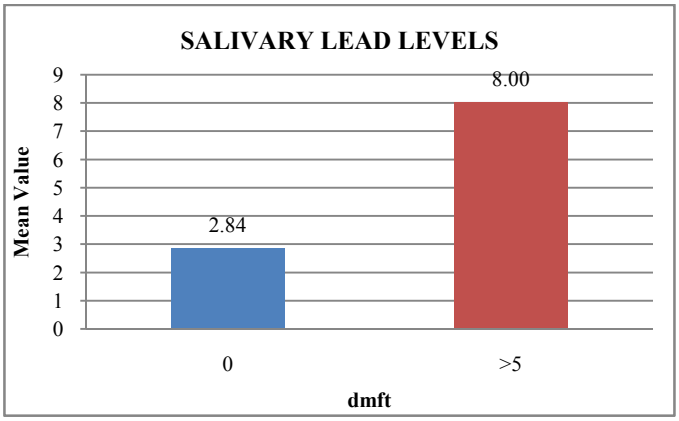


**Figure 3** Comparison of salivary lead levels in both the groups

Certain studies were carried out to assess the role of lead in dental caries. Moss [16] used data from the NHANES (The National Health and Nutrition Examination Survey) III collected from 1988 to 1994, and suggested that the attributable risk of lead exposure in the population is estimated to be 13.5 percent for dental caries. However, the present study was conducted with an aim to find out correlation between salivary lead and dental caries in population of Ahmedabad city by estimation of salivary lead in 100 children who were divided in study and control group based on dmft index. The result of this study showed nearly 3 times more salivary lead level in study group than control group with p value less than 0.001, suggesting that there is a positive correlation between salivary lead level and prevalence of dental caries.

F. Gil *et al* (1996) conducted a study to assess the relationships of lead content in teeth with the prevalence of caries, dental plaque, pH of saliva, levels of Salivalis Lactobacilli (SL) and Mutans Streptococci (MS), degree of dental abrasion, tooth color and tooth brushing frequency and concluded that accumulation of lead in teeth is associated with these factors. Highest number of colonies of SL and MS were found in saliva with the highest lead tooth levels [19].

Other biomarkers that can be used to find out lead exposure of human body are blood, sweat, nail, hair, teeth, bone, urine and faeces [20]. Blood is the most commonly used method to assess both occupational and environmental exposures to inorganic lead. However, the invasive nature of blood sample collection from children and the elderly, requirement of trained phlebotomists and many other factors like sample transport/storage and ethical approval issues make blood less ideal for human biomonitoring, more particularly in large population surveys [21]. Hence saliva has been suggested as a good monitor for recent lead exposure [1]. In the study of Nriagu (2005) [22], Sroisiri *et al* (2005)[23], Jackie Morton *et al* (2013) [21] and similar other studies, salivary and blood lead level is determined and they concluded that there is a weak relationship between salivary and blood lead level. These studies showing weak correlation had a consistent finding that salivary lead is 8 to 10 times less than blood lead level. It may be because the lead in saliva is derived from plasma fraction of blood and that it is not related to the bound fraction[24].

The most common laboratory methods available to determine lead concentrations are Atomic Absorption Spectrometry (AAS), Anodic Stripping Voltammetry (ASV) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). AAS is based on the concept that free atoms absorb light at wavelengths characteristic of the particular element. Flame Atomic Absorption Spectrometry (FAAS) has limit of detection in the order of 10-30 mcg/dl, while that of Graphite Furnace Atomic Absorption Spectrometry (GFAAS) is in the order of 1–2 mcg/dl. The detection limit of ICP-MS method for direct analysis of lead in blood is approximately 0.1 mcg/dl [20]. In saliva, only a fraction of unbound plasma lead from blood is present [22]; so lead level is in very small quantity and therefore ICP-MS with lowest detection limit was the choice of laboratory method used in this study.

Result of the present study are in accordance with the study of Shaimaa Hamid Mudherii *et al* (2010)[25] which checked correlation between salivary and blood lead level in lead exposed and unexposed population and dental caries. Result of the study of Nattaporn Youravong *et al* (2013) [26] contradicted result of this study which showed no significant association between salivary lead level and dental caries.

In this study, means of control and study groups are 2.84 and 8.00 mcg/L respectively. These all figures increase concern about probability of high lead exposure in Ahmedabad city. In the last two decades, there are rapidly increasing government projects and industrialization (e.g. petrochemical, tile industry) as well as the number of vehicles in Ahmedabad city which may result in increased lead in the environment and may be a reason for high salivary lead level in this study.

There are few studies related to lead level and caries conducted in India. Shashikiran ND *et al* (2007) [1] carried out a study in Davangere city. Another study was conducted in 2010 in Dariba[18], Rajasthan, India. The results showed that lead level in study population was above 'level of concern' as given by the Centers for Disease Control (CDC)[9]. In the present study lead concentration was high in patients with more number of carious lesions. If lead level is higher than the level of concern, preventive measures should be implemented by state government at public health level. Specific strategies that aim at screening of high-risk children are necessary to identify children with elevated lead level. Once identified, children with elevated lead levels should be given follow-up services and primary prevention protocol should be planned in such contaminated areas.

## CONCLUSION

The mean salivary lead level of study group is nearly thrice of that found in the control group samples. However control group also shows higher values of lead in few samples. Hence, it can be concluded that salivary lead cannot be the only etiologic factor for the prevalence of dental caries, but it can significantly influence the susceptibility of dental caries. Another important conclusion of this study is that saliva may be a good alternative for biomonitoring of lead especially in screening programs. It is necessary to decide a proper guideline and universal criteria for saliva collection method and sample transport to use this noninvasive biomarker.

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