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RESEARCH ARTICLE

ASSOCIATION OF IDIOMATIC SALIVARY AND BLOOD LEAD LEVELS AS BIOMARKERS OF DENTAL CARIES IN PRIMARY DENTITION

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ABSTRACT

Aim: The purpose of the study was to examine the correlation between individual's salivary, blood lead levels 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 study and control group. Samples of unstimulated saliva and blood 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. Also, blood lead level showed a positive correlation with dental caries.
Conclusion: The salivary and blood lead levels were found to be higher in subjects with dental caries proving the cariogenic potential of lead. Saliva proved to be a better biomarker than blood in analyzing the lead levels.
Clinical significance: Pediatric dentist should be aware of environmental pollutants like lead that can adversely affect general and dental health. There is also scope for future research to identify those mixtures of micro-minerals in food or water supplies that exert a cariogenic effect.

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INTRODUCTION

Role of trace elements in the development of dental caries has been an area of interest since the identification of the protective effects of fluoride (Shashikiran, 2007). 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 (BE Davies, 1987). 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 toxic and chronic effects on many tissues and accumulates in teeth and bones (Constance Wienera, 2015). There are many animal and human studies which support the concept that lead is a caries promoting element . 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 (Janicka, 2015 and Khandekar, 1978). Lead is a relatively heavy metal with atomic number 82 and atomic mass of 207.2 (Simons, 1989).

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Anglo-Saxon coined the word Lead**.** It is chemically symbolized as Pb and comes from the Latin word Plumbum**.** It is a widely used metal, since 5000 BC 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 7. Significant exposure to lead is an environmental threat to optimal health and to physical development in young children that affects all socioeconomic groups. The most susceptible populations are particularly toddlers and infants in the neonatal period because of their hand to mouth practice. Lead enters our body through two different routes: inhalation and ingestion (Bercovitz, 1990). Sources of lead are paints, painted toys, folk medicines, ayurvedic medicines, gasoline additives, cosmetics, lead glazed ceramics, dust, and potteries. Besides the settling .of atmospheric lead, surface contamination also occurs from contact with industrial waste containing lead (Nikolas, 2005). In dentistry, sources of lead are metal brackets, orthodontic appliances and intraoral x-ray films (Olmedo, 2010). 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 used in the mouths of patients. This is important because inorganic lead is easily dissolved in human saliv (Leonard, 2005). Plasma, serum, saliva, bone, hair, nail, urine and feces can be used as a biomarker of lead instead of blood [12]. 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 (Fernando Barbosa Jr., 2005). There is hardly any published data regarding the association of blood lead levels, salivary lead levels and dental caries in the Indian literature. Hence this study was carried out to explore the possible correlation between blood and salivary lead levels and dental caries.

MATERIALS AND METHODS

Children of age group of 3-6 years, visiting the Department of Pedodontics and Preventive Dentistry of Government Dental College and Hospital, Ahmedabad were included in this study. The study was carried out after obtaining ethical clearance from 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 caries group with dmft score more than 5. In both the groups, unstimulated saliva as well as blood samples were 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, who were willing to give their blood and salivary sample. 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. Each child received a thorough dental clinical examination for dental caries by the same investigator, according to a strict, well tested protocol (WHO criteria for assessment of dental caries). 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 and by same examiner. 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.

Method of blood sample collection: Blood was drawn with the help of disposable syringes, equipped with stainless steel tips. Venous blood sample was taken from cubital vein. Blood was collected in green vacutainers tube containing 1 mg/ml of lithium heparin (diluents) and was stored in the refrigerator at 4 to 6°C till further analysis. Lead estimation of the samples done by ICP-MS (Make: Agilent 7700 MS [2013]) was performed 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 blood and saliva sample 2. Lead estimation in blood and 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. Both saliva and blood samples were prepared for analysis of lead. The 2 ml saliva sample was digested using 3ml of 50% nitric acid and the sample was diluted to 5 ml ultra purified water with the help of micropipette. With help of micropipette 1 ml of blood sample was taken into the container then digested using 4 ml of 50% nitric acid and the sample was diluted with 5 ml ultra purified water. 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 estimated using ICP-MS.

Lead estimation by ICP-MS

Elements were digested in 4% nitric acid solution. Analytes in solution were 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 and blood samples were introduced and lead concentration values were recorded in computerized system. Unpaired student-t test was used for statistical analysis, with the level of significance set at 0.05.

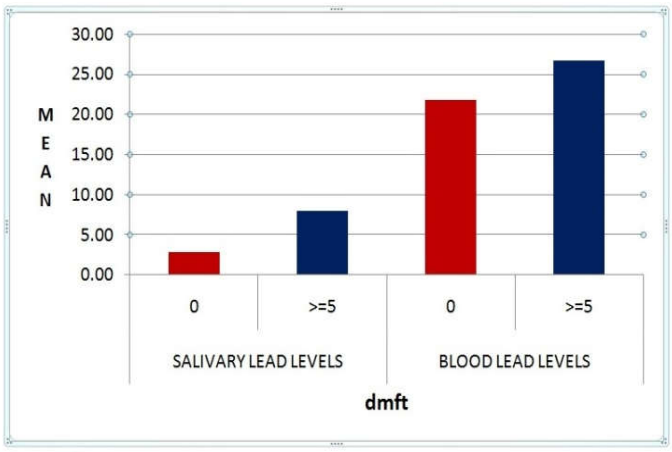

Figure 1. Bar Diagram showing mean salivary and blood lead values in both study and control groups

RESULTS

A highly significant correlation was found between salivary lead level and the presence of dental caries. Also, blood lead level showed a positive correlation with dental caries. In both the samples, the p value was found to be < 0.005 (Table 1, 2 and 3).

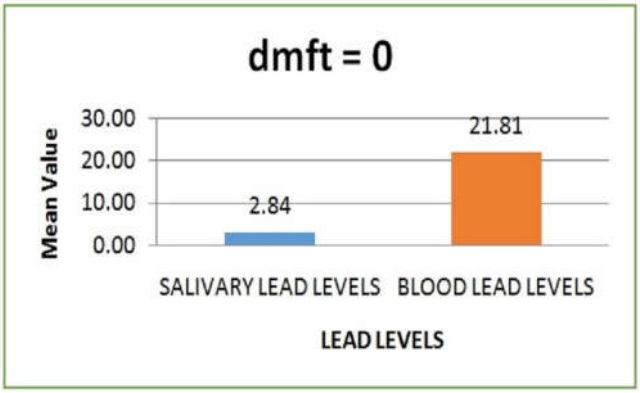

Figure 2. Comparison of salivary and blood lead levels in the control group

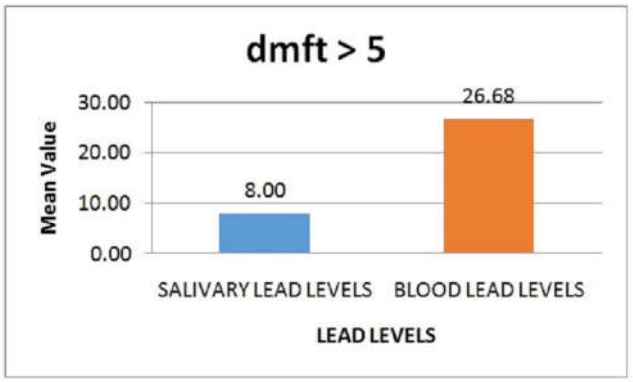

Figure 3. Comparison of salivary and blood lead levels in the study group

Table 1. Showing statistical analysis results of control group and study group

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		
Blood lead levels	0	100	21.81	12.38	1.238	-4.86	0.004
	>=5	100	26.68	11.25	1.125		

Table 2. Salivary and blood lead levels in the control group

dmft 0	N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	P Value
Salivary Lead Level	100	2.84	2.02	.202	-18.97	<0.001
Blood Lead Level	100	21.81	12.38	1.238		

Table 3. Salivary and blood lead levels in the study group

dmft > 5	N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	P Value
Salivary lead level	100	8.00	3.92	.392	-18.67	<0.001
Blood lead level	100	26.68	11.25	1.125		

Table 4. Showing positive correlation between blood and salivary lead levels and dental caries (dmft)

Linear Regression

N	Correlation	P Value	Result	Linear Regression	R Square
200	0.202	0.004	Partial Positive Correlation	dmft=0.008*blood lead levels+1.296	.041
200	0.640	<0.001	Perfect Positive Correlation	dmft=0.079*salivary lead levels+1.070	.409

Subjects with dmft 0 (control group) had a mean salivary lead level of 2.84 and a mean blood lead level of 21.81 (Figure I). Subjects with dmft > 5 (study group) had a mean salivary lead level of 8.00 and a mean blood lead level of 26.68 (Figure I). Figure II and Figure III shows comparison between blood and salivary lead levels in both study and control group.

DISCUSSION

Human activities and extensive use of lead in industry have resulted in its redistribution leading to contamination of air, water and food resulting in significant rise in lead concentration in human blood and body organs especially in a developing country like India (Sapna Hegde, 2010). The severity of lead toxicity depends on the duration, frequency, and amount of exposure. Lead poisoning usually results from cumulative absorption of small amounts of lead in the body. When lead enters the blood it can cause serious health problems in adults. There are a number of studies conducted in developed countries to check minimum threshold level of lead as well as adverse health effects of lead. They also started primary and secondary preventive measures for elevated lead levels. There is scarcity of data in India for a lead level in different cities, even though there is increasing level of pollution rapidly in last two decades. This study was a small attempt to check lead levels in population of Ahmedabad city, one of the most polluted cities of India. Blood is the most commonly used and gold standard method to assess both occupational and environmental exposures to inorganic lead. However, the invasive and traumatic nature of blood sampling from children and the elder, need for trained phlebotomists and many other factors including sample transport/storage and ethical approval issues make blood far from ideal for human biomonitoring, particularly in large general population surveys. Saliva has been suggested as a good monitor for recent lead exposure (Shashikiran, 2007). In healthy individuals, the resting salivary flow rate typically is 0.3–0.7 ml/min and average

saliva production has been estimated to be 1.6 L/day (Jerome Nriagu, 2006). These all figures suggest that saliva may be a significant route in the accumulation of lead on the enamel. So in the present study, saliva was also chosen as a biomarker for lead estimation. Many studies have been done to check the correlation of blood (PbB) and salivary lead levels (PbS). In study of Nriagu (2005)[15], Jackie Morton (2013) and many other studies, salivary and blood lead levels were checked and they concluded the same 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 approaches from plasma fraction of blood and that it does not relate to the bound fraction (Viviane Elisângela Gomes, 2004). In the present study, lead level in saliva is present in both control and study group with mean PbS 2.84 mcg/L and 8.0 mcg/L respectively. Mean of salivary lead level of study group was 3 times higher than salivary lead in the control group, with p value less than 0.05 in present study showing positive relation between caries and lead. As dental caries is a multifactorial chronic disease which must require other etiologic factors –diet, microorganism, host and time. Lead is also present in control group with maximum 9.28mcg/L with patient having dmft+DMFT zero. So even though there is statistically significant mean difference, it cannot be concluded that lead directly increases the prevalence of dental caries.

Lead may be a trace element which increases susceptibility to dental caries. In the present study, the concentrations of lead in blood (PbB) for both groups ranged from values 4.06 mcg/l to 48.55 mcg/l; where blood lead level ranges from 4.06 to 45 mcg /l with mean of 21.81 mcg /l in control group and in study group, the blood lead level ranged from 11.9 mcg /l to 48.55 mcg /l with mean of 26.68 mcg /l. It is difficult to explain role of lead in caries as causal or risk factor. This shows that lead increases caries susceptibility and plays a pivotal role in multifactorial process of caries. This also shows that blood lead is not directly proven to be cariogenic but it provides proxy for other cariogenic factors. There are few studies conducted in India related with lead level and caries. In 2015, Shashikiran ND (Shashikiran, 2007) conducted a study in Davangere city, India. The results showed that lead level in population is above the 'level of concern' as given by the CDC. In present study lead concentration is high in patients with more carious lesions. If lead level is higher than level of concern, preventive measures should be taken by state government at public health level. Specific strategies that target screening of high-risk children are essential to identify children with elevated lead level. Once identified, children with elevated lead levels should receive follow-up services and primary prevention protocol should be prepared in such contaminated areas.

Conclusion

The present study concludes that saliva is a definite biomarker for recent body lead burden and there is definite relation between blood lead level, salivary lead levels and dental caries. However, further in depth study on larger scale is required to establish the acceptable blood and salivary lead levels in Indian population. More studies are required to establish that lead is risk factor or etiological factor for dental caries.

Clinical Significance

The dentist should educate the parents regarding the sources of lead and adequate measures to prevent its exposure, for better oral and general health of the child.The role of trace elements like lead in the causation of dental caries should not be underestimated by the dentist. A major advantage of conducting research in communities is that the study findings often have immediate relevance and policy implications for the community. Pediatric dentist should also be aware of environmental pollutants like lead that can adversely affect general and dental health. There is also scope for future research to identify those mixtures of micro-minerals in food or water supplies that exert a cariogenic effect.

Conflicts of Interest: There are no conflicts of interest for the present research.

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