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Research Article

Blood Lead Level As A Biomarker For Dental Caries – A Transverse Clinical Study

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ABSTRACT

Purpose of the study: The purpose of the study was to assess the association between blood 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 (dmf=0) and study group (dmf>5)**.** Samples of 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 partial positive correlation was found between blood lead level and the presence of dental caries. The average values of lead in the control and study group were 21.81 mcg/l and 26.68 mcg/l, respectively.
Conclusion: The blood lead levels were found to be higher in subjects with dental caries proving the cariogenic potential of lead.

INTRODUCTION:

Since the identification of the protective effects of fluoride, the role of other trace elements in the development of dental caries has been an area of interest [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 toxic and chronic effects on many tissues and accumulates in teeth and bones [3]. There are many animal and human researches 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 has an atomic number 82 and atomic mass of 207.2 [7]. Anglo-Saxon coined the word Lead. It is chemically symbolized as Pb and comes from the Latin word Plumbum. It is a highly used metal, 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. 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 socioeconomic groups[9].The foremost inclined populations are significantly toddlers and infants owing to 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].

Dental sources of lead are 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 used 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 [16]. 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 insignificant published data regarding the association of blood lead level and dental caries in the Indian literature. Hence this study was carried out to explore the possible association between idiomatic blood lead level and dental caries.

MATERIALS AND METHOD:

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 approved by the Institutional Ethical Committee. The study sample was equally divided into following two groups of 100 each: Group A consisting of 100 children of control group with dmf score '0' and Group B consisting of 100 children of study group with dmf score more than 5. In both the groups, blood samples were collected. Informed consent was obtained from parents/guardians of all children included in this study.In this study, children who were willing to give their blood 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.Each child received a thorough dental clinical examination for dental caries by two qualified dental personnel, 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 blood sample collection:

Blood was drawn with the help of disposable syringes, equipped with stainless steel tips by trained phlebotomists and pathologists. Venous blood sample was taken from cubital vein. Minimum 3ml was required. Blood was collected in green vacutainers tube containing 1 mg/ml of lithium heparin (diluents). Blood samples were stored in the refrigerator at 4 to 6°C till further analysis. The vacutainers with the blood sample were capped, placed in ice bag and stored in the refrigerator until transported to the laboratory in an ice box at the end of each day.

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 blood sample
2. Lead estimation in blood sample by ICP-MS

1. Digestion procedure

All glassware and plastic ware were immersed in nitric acid overnight and rinsed with ultra purified water to eliminate lead contamination. Blood samples were prepared for analysis of lead. 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 detected using ICP-MS.

2. 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 blood 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).Blood lead level showed a positive correlation with the occurrence of dental caries with p value of 0.004 (Table 3, figure 3). Subjects with dmft 0 had a mean blood lead level of 21.81(Table 1, Figure 1) while those with dmft > 5 had a mean blood lead level of 26.68 (Table 2, Figure 2).

Table 1. Blood lead levels in the control group

Blood Levels	Lead	N	Mean	Std. Deviation	Std. Error Mean
dmft	0	100	21.81	12.38	1.238

Table 2. Blood lead levels in the study group

Blood Levels	LEAD	N	Mean	Std. Deviation	Std. Error Mean
dmft >5	0	100	26.68	11.25	1.125

Table 3. Blood lead levels in both the groups

Blood Levels	Lead	N	Mean	Std. Deviation	Std. Error Mean	Mean Difference	P Value
dmft	0	100	21.81	12.38	1.238	-4.86	0.004
	>5	100	26.68	11.25	1.125		

Figure 1. Blood lead levels in the control group

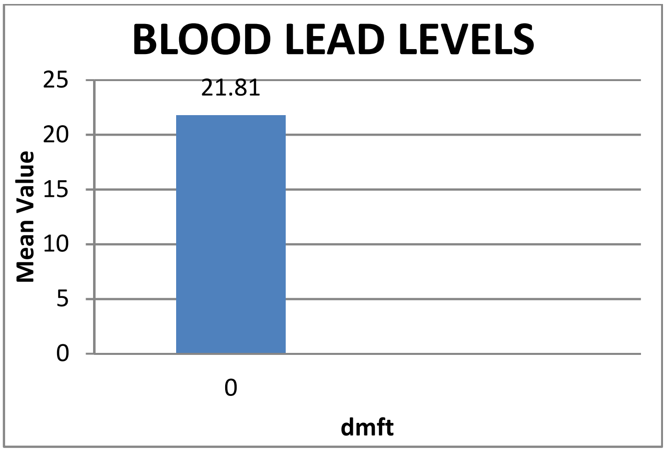


Figure 2. Blood lead levels in the study control group

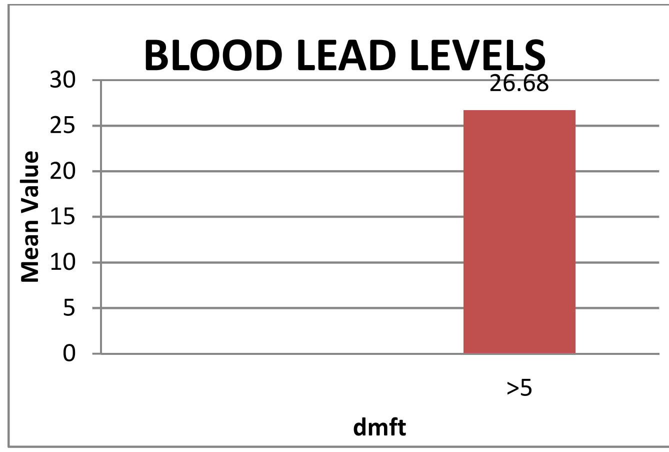
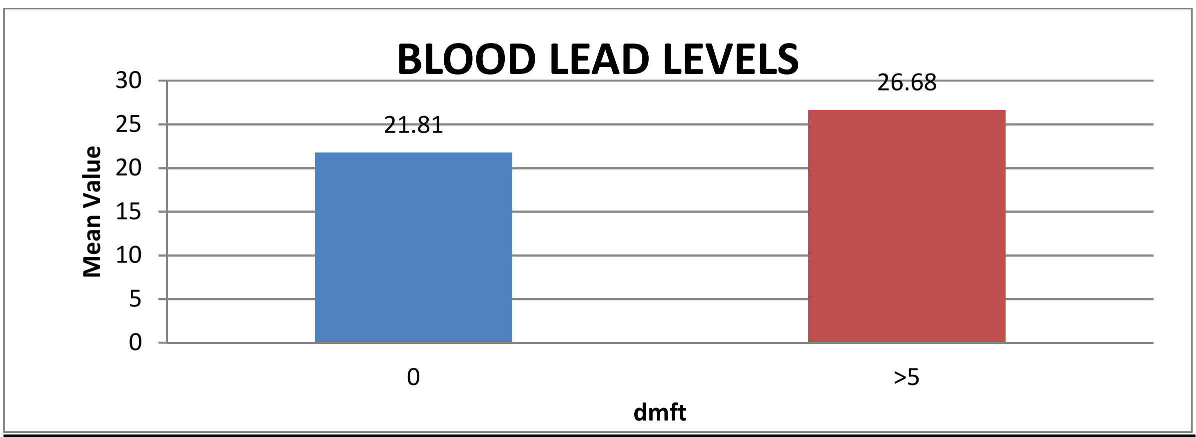


Figure 3. Comparison of blood lead levels in both the groups



DISCUSSION:

India is a rapidly developing country with increase in commercialization of various regions and cities of which Ahmedabad is one such region. With the increase in environmental pollution, the concentration of trace element such as lead also increases. The sources of lead in this region are industries like petrochemicals, chemicals and fertilizers, tile industry, increase in the use of vehicles, increased consumption of sea food, houses with peeling lead-based paint chips, etc. All these factors increase the total daily exposure of children to lead. Thus, this study was carried out to assess the exposure of lead in Ahmedabad city on a smaller scale. Due to significant lack of data related to blood lead level and dental caries in Indian population, it is necessary that data be collected, correlated and compared with that of different populations. There was lack of such data seen in the children from Ahmedabad. All these facts contributed to the need to carry out this study in the children of Ahmedabad to estimate the blood lead level and dental caries prevalence.

As per WHO norms permissible lead level in blood is 40 mcg/dl (400 mcg/l) for adults and 25 mcg/dl (250 mcg/l) for children, though it should be zero.[17] The margin of safety with lead is very narrow. The Centers for Disease Control has lowered the acceptable concentration of lead in blood in young children from < 25 mcg/dl to < 10 mcg/dl [18]. In this study blood lead level ranges from 4.06 mcg /l to 48.55 mcg/l, which is less than the harmful dose. In the present study, it was observed that all the children included in the control group had varied amounts of lead which clearly indicates lead pollution from the environment among all growing children of Ahmedabad. In this study, in the control group, blood lead level ranged from 4.06 to 45.29 mcg/l with mean of 21.81 mcg/l.

Blood is a useful biomarkers for monitoring the exposure and effects of lead in human population.[19] Lead in blood has half-life of 30 days and it reflects recent exposure.[20] Within the blood compartment, lead is divided between red blood cells (RBCs) and plasma, with RBCs accounting for 95% of the blood lead burden. It is then redistributed to bone (~ 70%) and soft tissues and then slowly excreted, with its biological half-life estimated at 10 years. Inspite of the fact that majority of lead present in blood is in red cells, plasma lead levels is thought to better reflect lead transferred from bone stores to target tissues. As red blood cells have limited capacity to accumulate lead, the relation of blood lead to plasma or serum lead is nonlinear, with serum lead increasing more rapidly at higher blood levels.

The determination of blood lead levels is not itself a measure of health. The study by Jawad Mehdi concluded that the levels of lead in deciduous teeth and blood serve as a dosimeter for lead exposure originating from various sources. Measurement of blood lead levels is the most accurate method of assessing the actual current exposure in individuals and populations.[21] Blood is the most universally accepted biomonitoring agent for lead estimation, however it is an invasive method, needs trained laboratory technician, there is difficulty in sample storage and it requires ethical clearance.[19] Current blood lead level is a good estimate of lead exposure during early childhood when teeth are being formed.[22]

Watson et al in their study, found that prenatal and perinatal exposure to lead resulted in a high rate of dental caries in laboratory rats, which approximates 40% [23]. Lead appears to be more concentrated in dental plaque than the surrounding saliva which is related to increased prevalence of caries as cycle between remineralization and demineralization is disrupted [22]. The results of study conducted by Shashikiran et al revealed the presence of 18 trace elements in the enamel of teeth of which one was lead [1]. On the basis of animal and human studies, Navia has summarized the cariogenic effect of various elements, of which lead was found to be a caries promoting mineral.[24]

Irving concluded that as compared to enamel, the levels of lead in secondary dentine are elevated because of the proximity of these zones to the vascular supply of the pulp.[25]

ICP-MS method is very sensitive for estimation of trace elements and also economical if estimation of more than one trace element is required. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a multi-element technique which utilizes an inductively coupled plasma (a very high temperature ionized gas composed of electrons and positively charged ions) source to atomize the sample and subsequently ionize the atoms of interest. The ions are extracted from the plasma and passed through a mass spectrometer, where they are segregated and measured according to their mass-to-charge ratio. It provides very low detection limits (parts per trillion (ppt) to parts per billion (ppb) for most trace elements. The detection limit of ICP-MS method for direct analysis of lead in blood is approximately 0.1 mcg/dl. ICP-MS is less tolerant than GFAAS (Graphite Furnace Atomic Absorption Spectrometry) to heavy matrices, so dilution of blood samples prior to aspiration into the plasma is required; therefore, ICP-MS method needs skilled laboratory technicians for operation at the highest standards.[26]

The presence of lead was detected both in the control group and the study group. But the lead levels increased with increasing caries prevalence. The present analysis supports the hypothesis that environmental lead exposure is a risk factor for dental caries. However, studies on larger sample size are required to be carried out to confirm the role of lead in caries prevalence. The concentrations of lead in blood for both groups ranged from 4.06 mcg/l to 48.55 mcg/l. In group A which is the control group, blood lead level ranges from 4.06 to 45.29 mcg /l with mean of 21.81 mcg /l. In group B which is the study group, blood lead level ranges from 11.9 mcg /l to 48.55 mcg /l with mean of 26.68 mcg /l. The results of the present study shows that there is positive correlation between caries prevalence and blood lead level in the study group. But higher blood lead levels (45 mcg /l) were also found in control group where caries prevalence was zero.

It is difficult to explain the role of lead as a risk factor for dental caries. It can be said that lead increases caries susceptibility and plays a vital role in the multifactorial carious process. It is believed that blood lead is not directly proved to be cariogenic but it provides proxy for other cariogenic factors. In this study p value is 0.004 (< 0.05) which means that lead level and caries has a significant relation. If correlation of environmental lead exposure and dental caries is proved, it would have considerable need to broaden the focus of health care interventions for dental caries apart from modifying dietary habits, improving personal oral hygiene methods, and increasing fluoride exposure in high-risk groups.[22]

The results of the present study suggest that environmental lead exposure may explain, at least in part, the disproportionately high rate of dental caries. The cross-sectional nature of data limits the ability to test mechanism-specific hypotheses. For example, if lead exposure at the time of enamel formation was the most common mechanism for a lead-caries causal association, it would be required to assume that current blood lead level is a good estimate of lead exposure during early childhood when teeth are being formed. In conclusion, these data suggest that blood lead levels are associated with dental caries in Ahmedabad.

CONCLUSION:

The results of this study showed that blood is definite biomarker for recent body lead burden. There is definite relation between blood lead level and dental caries. however, further in depth study on larger scale is required to establish the acceptable blood lead level in Indian population. More studies are required to establish that lead is risk factor or etiological factor for dental caries. Also, 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.

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