TRACE ELEMENTAL ANALYSIS OF DENTAL CARIES IN HUMAN TEETH BY EXTERNAL PIXE

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ABSTRACT: The elemental profiles of the dental caries in human teeth were analyzed by the external proton induced X-ray emission (External PIXE) studies. A total of ten elements including trace namely P, Ca, V, Mn, Fe, Cu, Zn, As, Sr and Pb were estimated in the present study. P and Ca were found to be the major elements whereas all other elements were in trace level. It was observed that the concentration of elements Phosphorus and Calcium varied between the range 7.98% and 19.26% and 19.83% and 35.2% respectively in dental caries.

Keywords: Dental caries; Trace element; External PIXE, Human teeth

INTRODUCTION

Dental caries the most prevalent childhood chronic disease in many countries is a major public health problem and is associated with preventable pain and high treatment costs. World wide most children and an estimated ninety percent of adults have experienced caries, with the disease most prevalent in African countries. In the United States, dental caries is the most common chronic childhood disease, being at least five times more common than asthma. It is the primary pathological cause of tooth loss in children. Between 29% and 59% of adults over the age of fifty experience caries. During the last decades, an important decrease in the prevalence and severity of dental caries was observed both in developed and developing countries such as Brazil, Iran, India and Srilanka etc.

Dental caries is preventable disease of the mineralized tissues of the teeth with a multi-factorial aetiology related to the interactions over time between tooth substance and certain microorganisms and dietary carbohydrates producing plaque acids. Dental caries is the result of complex interactions involving the individual (nutrition, genetics, behavior, race and age), plaque bacteria, saliva flow and composition of the environment. Elements play an important role in human health. Excess and deficiency of these, resulting from exposure to both the natural and man made environment, can lead to a wide variety of clinical effects (Brown, et.al., 2004). Teeth are reported to be suitable indicators of trace element exposure for a wide range of elements. Since teeth accumulate a variety of trace elements, it is very interesting to study the elemental distribution in human teeth to evaluate biological processes. The knowledge of the spatial distribution of trace elements in tissues is involved in many biological functions of living organisms. In this way, elemental distribution in teeth can provide information about physiology of elements, environmental influence, contamination by metallic amalgams used as restorative materials and dietary habits (Anjos, et.al., 2004)
Non-destructive analyses of the major and trace elements present in biological samples are of great importance, because these materials are sometimes too precious to be analyzed destructively. Ion beam techniques have been widely employed in dentistry and their sensitivity for trace elements in various thick tissue specimens was attentively investigated (Bennun, et.al., 2002; Beck, 2005; Ryan, et.al., 2005; Pallon, et.al., 2005). Teeth are not homogeneous in elemental composition as demonstrated in earlier studies (Evans, et.al., 1995; Carvalho, et.al., 2000; Carvalho, et.al., 2004) and consequently thick targets of dental caries were used for analysis.

It has been reported that the concentration of Pb in teeth can be used as an index of environmental pollution (Frank, et.al., 1990; Bercovitz and Laufer, et.al., 1992; Gulson and Wilson, et.al., 1994). Lead is preferentially incorporated and stored in calcified tissue such as teeth (Begerow, et.al., 1994).

Among various analytical techniques, atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS) are becoming more routine, but they are destructive chemical methods involving sample dissolution procedure and their inability for analysis of samples in solid form. The electron probe micro analyzer (EPMA) technique is useful for the analysis of even a mono-mineral grain, but is limited to detection of elements at concentration more than 200 ppm. In this context, multi elemental non-destructive accelerator based external proton induced X-ray emission (external PIXE) technique looks more useful for elemental analysis of samples. It is fast, simultaneous, reliable, quantitative, multi-elemental and non-destructive with an excellent sensitivity in the ppm level and detection limits across a wide range of atomic numbers (Nayak, et.al., 2004). On the other hand, the samples can be irradiated as such in air which is extremely useful in analyzing any specific area of the samples. For all of these advantages, external PIXE technique was preferred for the present study for elemental analysis of the dental caries samples of human teeth.

MATERIALS AND METHODS

2.1 Sample collection and processing

Human teeth carious samples were collected from the Anil Neerukonda NRI General Hospital, Sangivalasa, Visakhapatnam, India from individuals in the age group of 33-57. Prior to the teeth extraction, a detailed investigation of dental history and examination were carried out for each individual. Immediately after the extraction each tooth was put into oxygenated water (to clean the organic material from its surface) into a separate polyethylene container. Later the samples were acid cleaned and washed with deionized water and then with alcohol. The specimens were sealed in individual plastic containers in deep freezer till the irradiation (at -20^0 C). To avoid the contamination of the samples, proper cares were taken for the collection of specimens and their handling prior to the analysis.

2.2 Experimental setup

The elemental analyses of the teeth samples were carried out in the external PIXE set-up at Institute of Physics, Bhubaneswar which is the unique of its kind in India and was installed by Vijayan et.al., (2003). The proton beam of 3 MeV energy obtained from the 3 MV tandem type horizontal pelletron accelerator (Model: 9SDH-2, make: National Electrostatics Corporation, Madison, USA) was used for irradiation of teeth. The proton beam was collimated by a graphite collimator to a beam size of 1-mm diameter. The beam was extracted into air using a Kapton^{TM} foil (8-μm thick) at the exit point of a vacuum scattering chamber (Prasad, 1995). The beam was first focused and centered at the target location inside the scattering chamber and then let through the thin Kapton foil placed at the exit port. The Kapton foil is used as exit window due to its several special characteristics like low beam-induced background emission, minimal energy loss and resistance to radiation damage. The beam was allowed to travel a few cm in air after which it irradiates the samples. Beam charge measurements were carried out by using a rotating vane chopper designed by Sahu, et.al., (2003). The samples along with the NIST (National Institute of Standards and Technology) Bone Ash international standard were irradiated with maximum beam current of 15nA. A Si (Li) detector (active area 30 mm^2) having energy resolution of 170 eV at 5.9 keV placed at 90^0 with respect to the beam direction was used to detect characteristic X-rays emitted from the target (Vijayan et. al., 2003). The detector has an entrance beryllium window of 8 μm thickness. A 25-μm thick aluminium absorber (with 6% hole) was kept in front of the detector to attenuate the bremsstrahlung background and the dominant low energy X-ray peaks (Choudhury, et. al., 2003). Spectra were recorded by using a PC based multi channel analyzer.

2.3 Data Analysis

The PIXE spectral analyses were performed using GUPIX- 2004 software. This provides a non-linear least square fitting of the spectrum, together with subsequent conversion of the fitted X-ray peak intensities into elemental concentrations, utilizing the fundamental parameter method (FPM) for quantitative analysis (Rautray, et.al., 2009). The uncertainty in the concentration estimation for the major elements is 0.1 to 0.3% for Ca and P whereas for other elements it is found to be between 5 and 12% (Campbell, et.al. 2000; Rautray, et.al. 2007).

RESULTS AND DISCUSSION

In 1921, major Fernando E.Rodriguez Vargas, DDS of the Army dental corps discovered the bacteria which causes dental caries. According to his investigation, three types of the Lactobacillus species, during the process of fermentation, are the causes of cavities. In December 1922, he published a fundamental work on the specific bacteriology of dental caries. His findings were published in the military dental journal titled "The specific study of the bacteriology of dental cavities". In the year 1928, Rodriguez Vargas developed the techniques and method of analyses for the study of dental caries and his work was published in the journal of the American Medical association. His findings include the effectiveness of iodine in addition to other chemical agents as disinfectants of the mucous membranes of the mouth. Since then, other scientists have used the findings of his investigations as the basis in the study of the bacteriology of dental caries.

It is now well accepted in dentistry that fluoride plays an inhibitory role in the development of dental caries. Curzon and Crocker have recently reported that the following elements may be important in either increasing or decreasing dental caries.

Caries inhibitory: Al. Fe, Se, Sr;

Caries promoting: Cu, Mn, Cd.

It has been shown that certain trace elements e.g. F, Fe, Zn and Pb are more concentrated in the surface enamel of teeth. In a series of pioneering studies, Ahlberg explored the applicability of particle induced x-ray emission technique analysis to dental materials. Particle induced x-ray emission (PIXE) and heavy ion elastic recoil detection analysis (HI-ERDA) are non-destructive analytical techniques and were used to measure the elementary concentrations in teeth and to evaluate changes of trace element concentration after a bleaching treatment products.

The mineral tissue of a tooth consists of hydroxyl-apatite (HAP) crystals $Ca_{10} (PO_4)_6(OH)_2$ and trace elements. Dental healthy status may be co-related to trace element concentrations and the presence of dental caries.

In the current study the set of dental caries of human teeth were carried out in the external PIXE set-up at Institute of Physics (IOP), Bhubaneswar, India. The external PIXE spectrum of the dental caries of a human tooth is given in Figure.1. A total of ten elements including trace viz P, Ca, V, Mn, Fe, Cu, Zn, As, Sr and Pb were estimated in the present study and their respective concentrations are given in Table.1. It was observed from Table.1, the concentration of P ranged between 7.98% and 19.26% whereas Ca ranged between 19.83% and 35.2%.

Since human teeth contains (HAP) hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$] as their primary inorganic constituent which contains calcium and phosphorous in it, hence the higher amount of Ca and P is obvious in these samples.

Table 1. Concentration (in ppm by weight unless specified) of elements in the dental caries portion of human teeth.

No	P (%)	Ca (%)	V	Mn	Fe	Cu	Zn	As	Sr	Pb
DC-1	12.43	28.23	11.2	6.5	52.09	11.5	35.1	6.1	56.7	9.6
DC-2	13.51	23.61	6.4	3.9	44.9	18.5	62.9	4.5	47.1	7.1
DC-3	7.98	26.81	8.5	4.1	64.23	15.9	53.6	8.2	63.4	12.6
DC-4	19.26	25.78	9.4	7.2	32.16	13.6	46.4	5.4	75.2	8.7
DC-5	13.44	29.94	7.9	6.4	26.8	17.5	58.5	9.8	39.5	13.5
DC-6	11.89	21.36	10.4	5.83	32.4	14.8	37.2	3.7	80.3	12.4
DC-7	8.84	35.2	6.9	4.8	59.3	16.3	49.8	4.3	61.6	10.3
DC-8	10.07	19.83	12.4	6.7	69.9	12.7	21.9	7.6	42.8	11.2

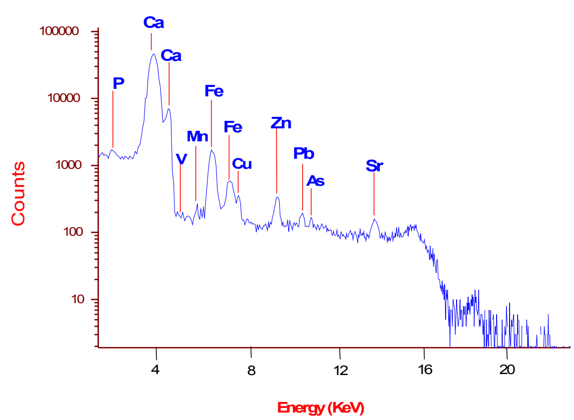

Figure. 1. The external PIXE spectrum of the dental caries of a human tooth

Concentration of V, Mn and Cu did not show much variation in all these samples.
Moreover, concentration of Fe, Cu, As and Pb in caries is higher than their normal levels. The accumulation of these elements on carious region may be from the type of food that is rich with these elements. The strontium content of teeth can provide information about the diet of past populations (Gilbert, 1985). Natural strontium levels in living bone and teeth are typically 150-200 ppm and in the present study, the concentrations of Sr in the carious areas of teeth are low. An interesting observation has been found that the Ca/Sr ratios are inversely proportional for dental caries of the teeth samples. However, the negative Ca and Sr correlation may be due to the fact that Ca is substituted by Sr either as a whole or in fractions for the formation of hydroxyapatite; therefore Ca and Sr are negatively correlated. Among the bivalent cations that can replace calcium in calcium hydroxyapatite, Sr has attracted a remarkable interest for its possible biological role. Sr is present in the mineral phase of the teeth, especially at the regions of high metabolic turn-over (Blake, et.al., 1986). The extent to which Sr is subject to diagenetic alteration is a matter of debate, though it is widely believed to be one of the least affected elements.
Pb can be toxic for humans and animals at some concentrations and it is a serious public health issue worldwide. Significant Pb contamination of ambient air, food, and wastewater provides exposure which will result in Pb accumulation in the teeth. Blood lead is an effective biomarker for lead exposure, for although lead also accumulates in bone, teeth and other tissues, the effects of lead on the human nervous system correlate with blood lead levels (Younes, 1995).
In the present study, it was observed that a few ppm of Pb is present in the human teeth samples whereas Pb concentration is high on the carious parts. This may be attributed to the accumulation of Pb directly from the food or water taken by the individuals. Lead toxicity directly and indirectly alters many aspects of bone cell function. Retention and mobilization of Pb in teeth occurs by the same mechanisms involved in regulating calcium influx and efflux, namely calcitonin and other hormones that influence calcium metabolism (Pounds, et.al., 1991).
Divalent cations such as Sr and Pb particularly interact and replace isovalent calcium sites in the hydroxyapatite in teeth matrix to form Sr- or Pb-substituted hydroxyapatite of dental tissue resulting in a permanent record that can indicate the past exposure (Fergusson and Purchase, 1987; Sharon, 1988). Hence, some parts of the total Sr or Pb measured in the teeth may be from the Sr and Pb-substituted hydroxyapatite. The estimated Sr or Pb concentration may be associated with Sr or Pb substituted hydroxyapatite present in teeth. The Zn concentrations in carious areas are considerably less than the remaining parts of the tooth sample.

Since Sr and Zn play major role in bone growth by increasing osteoblasts and decrease the number and the activity of osteoblasts, hence the carious areas showed depletion in Zn and Sr concentrations. Zn is naturally present in bone (Jallot, et.al., 1999) and stimulates bone growth and bone mineralization (Yamaguchi, et.al., 1986; Yamaguchi, et.al., 1987). Zn has a direct effect on osteoblastic cells in vitro (Hashizume and Yamaguchi, 1993) and a potent inhibitory effect on osteoclatic bone resorption (Kishi and Yamaguchi 1994). But, the clinical features of severe zinc deficiency in humans are growth retardation, delayed sexual and bone maturation, skin lesions, diarrhoea, alopecia, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral changes (Hambidge, 1987). In conditions of bone resorption and tissue catabolism, zinc is released and may be reutilized to some extent. Zinc is also involved in the general metabolism of humans so that relations may exist between dietary intake, diseases or occupational exposure and the bone mineral composition.

CONCLUSION

From the nondestructive elemental analysis of different parts of the teeth, it was clear that the elemental patterns in the carious part of the teeth are severely affected. The possible absorption of trace elements from food intake was observed. The present investigation is a preliminary attempt towards the specific identification of elemental role towards dental caries. Ca and P were found to be the major elements whereas all other elements were found in trace level in this study. It is planned to study the role of other elements by exposing the teeth samples in different parts to beam. Identification of other influential elements and factors are also planned to investigate in future. Hence this non-destructive external PIXE technique for the elemental analysis of materials is perfectly suited for the trace elemental analysis of teeth and dental caries of human being.

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