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Research Article

ELEMENTAL ANALYSIS OF DENTAL CALCULUS AMONG GUJARATI AND KERALA POPULATION FOR ESTABLISHING THE ASSOSCIATION OF DIETARY HABITS A

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A B S T R A C T

Dental calculus is formed by deposition of minerals from saliva and gingival crevicular fluids on to the teeth. Environmental conditions, dietary habits and lifestyle habits influences the oral environment and composition of dental calculus. Depending on the population these habits may vary .A study on the composition of dental calculus and the factors influencing its formation may help in the identification of these habits, which also varies according to the geographical location. A probable identification of a person based on the geographical location and lifestyle habits is possible by analysing the dental calculus. This study focuses on the variations in elemental composition between two population groups of Gujarat and Kerala and using it as a prospective tool in the identification of a person based on his geographical location and lifestyle habits.

INTRODUCTION

"Forensic odontology is the proper handling, examination and evaluation of dental evidence, which will be then presented in the interest of justice" (A.D.Goldman, 1982). Identification of individuals based on their dental parameters play an important role in situations of mass disasters. In cases of mass disasters, there is a wide probability of getting mixed population groups. Identification of persons based on their geography and life style habits, helps in the probable establishment of a person's identity. Hard tissues like teeth and dental calculus are well preserved within the oral cavity and can be easily used in personal identification, when most of the other tissues may be destroyed in situations like aviation disasters. Levels of calculus and its formation are population specific and are affected by oral hygiene habits, access to professional care, diet, age, ethnic origin, systemic diseases, and use of medication, habits like smoking, and other life style habits (White, 1997). Environmental conditions, dietary habits and lifestyle habits influences the oral environment and composition of dental calculus. This study focuses on the variations in elemental composition between two population groups and using it as a prospective tool in the identification of a person based on his geographical location and lifestyle habits.

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Aim and Objectives of the Study

To evaluate the difference in the elemental composition of dental calculus among the Gujarat and Kerala population and to use it as a prospective aid in the identification of geographical location, dietary habits and tobacco usage habits.

- To determine the elemental composition of dental calculus, both qualitatively and quantitatively in the population groups of Gujarat and Kerala with respect to the different dietary habit and tobacco usage habit
- To study the differences in the elemental composition of calculus among Gujarati and Kerala population with respect to the habits, lifestyles and to do a comparison grading of the elements.
- To establish the possible influence of geographical variation in the elemental composition of dental calculus.
- To detect the presence of toxic and trace elements in the dental calculus which may be a part of the formulations of different tobacco products or as may be possible with the dietary habits of chronic fish or meat eating.
- To establish the possible elevation in the levels of macro minerals and a depletion/ reduction in the micro minerals and vice versa due to the different habits across the two populations.

MATERIALS AND METHODS

Samples for this work consist of dental calculus collected from the two population groups of Kerala and Gujarat. Samples from Gujarat population were collected from the patients of department of Periodontology, AMC Dental College and

Hospital, Khokhara, Ahmedabad with the ethical committee approval. Samples from Kerala population was collected from the department of Periodontology, Amrita school of dentistry, Kochi.Small flecks of dental calculus were extracted from the patients following a brief history and with informed consent.

Inclusion Criteria

Age groups of 18 – 65 yrs.
Healthy individuals

Exclusion Criteria

Medically compromised patients
Patients with systemic diseases and under medications
Pregnant women and lactating mother
Patients with severe periodontal conditions or other oral diseases

A total of 80 samples from Gujarat and 66 samples from Kerala was collected with a detailed history of lifestyle habits and with informed consent. Samples collected were grouped into different categories based on the dietary and tobacco usage history

1. Gujarat Standard Samples (STN): Dental calculus samples collected from people without any history of tobacco usage were categorised as standard samples. (26 samples)
2. Gujarat Tobacco Smokers (T S) : Dental calculus samples collected from people with any kind of tobacco smoking habit were categorised as tobacco smokers and labelled as TS.(18 samples)
3. Gujarat Tobacco Chewers (T C) : Dental calculus samples collected from people with paan chewing, arecanut chewing, paan masala users, and other kinds of tobacco chewing habits were categorised as tobacco chewers and labelled as TC.(45 samples)
4. Kerala Vegetarians : Dental calculus samples collected from Kerala population who followed a vegetarian diet were categorised as Kerala vegetarians and labelled as K.veg – 14 samples
5. Kerala Non vegetarians : Dental calculus samples collected from Kerala population who followed a mixed dietary habit were categorised as Kerala Non vegetarians and labelled as K.non veg -52 samples

The dental calculus samples were collected with the use of sterilized hand scalers and personal protective equipments. Supragingival and subgingival calculus was collected from the lower anterior region with hand scalers. Samples were collected in plastic container and dipped in 70% ethanol for 12 hours and then air dried. Air tight and properly labelled plastic containers were used which allowed easy handling and transportation of the samples along with infection control.

The samples were subjected to non-destructive analysis using EDXRF. EDXRF is designed to analyse groups of elements simultaneously using the principle of X ray fluorescence. Qualitative and quantitative analysis of the elements is done using this instrument.

5 samples were analysed for elemental composition using SEM EDS.2 samples analysed for heavy metals using DP Voltammetry technique using MME pro.

Statistical analysis: SPSS software was used for the statistical analysis of the results.

RESULTS

Mean % Of Different Elements In Gujarat And Kerala And P Value

Elements	Gujarat Population	Kerala Population	p value
Calcium	73.96	72	0.008*
Phosphorous	22.48	23.45	0.03*
Strontium	0.088	0.042	0*
Iron	0.215	0.115	0*
Sulphur	2.65	1.86	0.034*
Potassium	1.677	1.045	0.013*
Zinc	0.105	0.081	0.036*
Nickel	0.006	0.004	0.76
Copper	0.111	0.073	0.002*
Titanium	0	1.465	
Silver	0.175	0	
Tin	0.139	0	

* P value significant

Mean % of Different Elements In Vegetarians And Mixed Dietary Groups And P Value

Elements	Vegetarians	Mixed dietary	p value
Calcium	70.686	73.844	0.015*
Phosphorous	22.47	23.99	0.023*
Strontium	0.028	0.045	0.035*
Iron	0.149	0.106	0.041*
Sulphur	3.04	1.62	0.039*
Potassium	1.2	0.985	0.303
Zinc	0.07	0.09	0.322

Mean % of Different Elements Among Tobacco Users And Non Users And P Value

Elements	Standards	Tobacco chewers	Tobacco smokers	P VALUE*
Calcium	74.158	70.36	73.53	0.013
Phosphorous	23.26	21.76	23.24	0.041
Strontium	0.084	0.113	0.07	0.041
Iron	0.152	0.263	0.154	0.012
Sulphur	1.52	3.21	1.21	0.001
Potassium	1.13	2.29	1.3	0.044
Zinc	0.079	0.126	0.109	0.047
Copper	0.084	0.134	0.073	0.018

*All p values significant after ANOVA test

Elemental Analysis of Pan Masala Products and Cigarrete Available In Market

PAN MASALA 1		PAN MASALA 2		CIGARRETE	
ELEMENT	%	ELEMENT	%	ELEMENT	%
Ca	53.301	Ca	91.189	Ca	60.18
Si	40.721	Fe	3.986	K	35.006
K	3.035	K	2.065	S	2.552
S	2.101	S	1.317	Fe	1.109
Fe	0.586	Sr	0.483	Mn	0.364
Cu	0.088	Mn	0.435	P	0.334
Mn	0.073	Br	0.225	Ti	0.192
Zn	0.056	Ti	0.221	Br	0.091
Br	0.025	Cu	0.081	Cu	0.087
Rb	0.014			Zn	0.066
				Rb	0.018

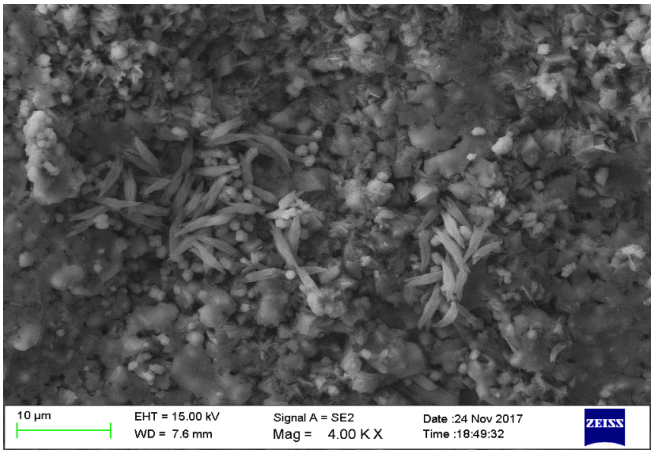
Sem EDS Analysis

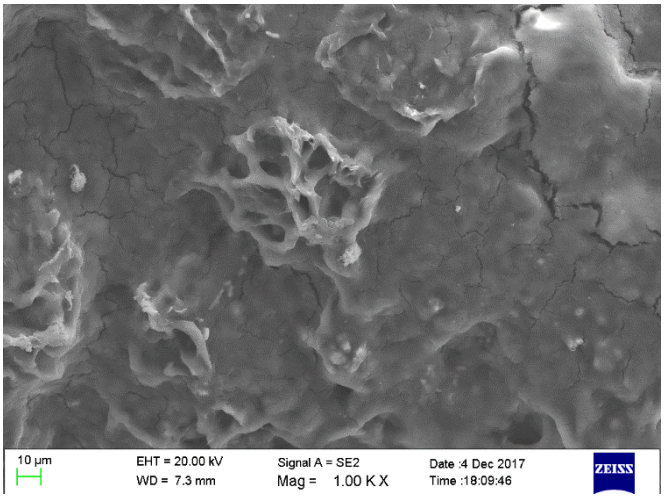
Scanning electron microscopic analysis of four dental calculus samples were done. SEM EDS analysis of the samples showed the presence of elements like carbon, oxygen, nitrogen, calcium, phosphorous, magnesium, fluoride and aluminium. Varying amounts of these elements are present in each samples. Elemental composition and the microstructure of the dental calculus samples depicts the crystalline nature of dental calculus. Major composition consists of calcium and phosphate hydroxyapatite crystals. Magnesium also present as part of the

crystalline structure. Fluoride also present as a constituent of the sample. Trace amounts of aluminium was also detected.

Voltammetry Analysis

Samples analysed for presence of heavy metals like Pb , As, by voltammetry. Only 2 samples were analysed and it showed presence of iron.





SEM Images of dental calculus

DISCUSSION

Calcium

The mean calcium concentration in Gujarat population is higher as compared to Kerala.

A higher calcium level in the Gujarat population in spite of majority being vegetarians, may be attributed to the increased intake of dairy products. Milk and milk products being a higher source of calcium. Milk has good bioavailability of calcium (about 30 – 35%) as compared to other sources(Weaver CM & Plawecki, 1994). Calcium in milk is more efficiently absorbed than any other calcium salts(Guéguen, 2000). A serving size of 8 oz of milk contains an estimated calcium amount of 300 mg.(National osteoporosis foundation). Gujarat tops in milk production. Per capita availability of milk in Gujarat during 2015-16 is 545 gm/day as compared to Kerala where it is 200 grams/day (National dairy development board) Monthly per capita quantity of consumption of milk (liquid) by State (Milk and Dairy Products in India – Production, Consumption and Exports- Anil Chawla, Hindustan Studies & Services Ltd.)

State	Monthly Per Capita Consumption (Rural)	Monthly Per Capita Consumption (Urban)
	Litres / Month	
Gujarat	4.975	6.702
Kerala	2.822	3.656

Calcium concentration in smokers and chewers were less compared to the standards.

This may be due to the reduced calcium concentrations in the saliva.Salivary flow and composition influence calculus formation. There are many studies showing a decreased level of calcium among smokers. In the study by Zuabi *et al* on subjects with established periodontitis, smokers exhibited greater disease level, but reduced sodium, calcium and magnesium concentrations.

In the study by Kolte *et al* on the effect of smoking on salivary composition, results showed reduced concentrations of total proteins, calcium, magnesium and phosphorus in whole saliva in smokers with chronic periodontitis. Similar results were seen in the study by Moghadam *et al*. In the study by Suresh *et al*, for the estimation of serum and salivary calcium levels in smokers and non smokers with chronic periodontitis, a reduced calcium levels were seen in smokers. An increase in the calcium levels were seen in males and females above 40 years.

Phosphorous

Phosphorous levels in Kerala population is higher as compared to Gujarat population. This may due to the increased intake of egg, meat, poultry and fish in Kerala. Within Kerala, people with vegetarian dietary habit showed lesser phosphorous levels as compared to the group with mixed dietary habit.

The two main sources of dietary phosphorus are organic, including animal and vegetarian proteins, and inorganic, mostly food preservatives. Phosphorous from animal protein is more absorbed than plant protein. About 40 to 60% of phosphorous is absorbed from animal proteinwhereas only 10 to 30 % is absorbed from plant protein. High phosphorous levels are present in fish. 3 ounces of fish (salmon) contains 282 mg of phosphorous, 8 ounces of milk contains 247 mg, 3 ounces of beef contains 173 mg, 3 ounces of chicken contains 155 mg. lesser phosphorous levels are present in plant based proteins. Phosphorous from plant seeds (beans, peas, cereals, nuts,) contain a non-protoplasmic storage form of phosphate and its bioavailability is less.

In Kerala population, dietary intake of fish, meat and poultry is high. The annual per capita consumption of fish in Kerala is very high (18.5 kg) as compared to the national average (5kg) (DEPT of fisheries, govt of Kerala).

High P levels in Kerala can thus be attributed to the high consumption of phosphorous rich animal protein. Certain food items like tamarind, spices like cardamom etc. are largely consumed in Kerala. Tamarind contains 628mg and cardamom contains 1119 mg of P /100 gm.

Phosphorous levels in the agricultural soils of Kerala is higher as compared to North western region of India. Nutrient index of phosphorous in agricultural soils of Kerala is 2.35 as compared to 1.64 of Gujarat, as estimated in 1997 (H Pathak ,2010- Indian Agricultural Research Institute).Thus the high phosphorus levels in Kerala can be established as a geographical marker

A depletion in the phosphorous levels of tobacco smokers and tobacco chewers were seen.In the study by Kolte *et al* on the

effect of smoking on salivary composition, results showed reduced concentrations of total proteins, calcium, magnesium and phosphorus in whole saliva in smokers with chronic periodontitis. (Kolte *et al* 2012).

Strontium

A higher concentration of strontium was seen in Gujarat population as compared to the Kerala population. This may be due to the increased intake of milk and dairy products in Gujarat, which are good sources of strontium. Pasteurized milk contains an average value of 0.86 mg of strontium per litre. Leafy vegetables, legumes and grains like wheat and barley are also rich sources of strontium and forms a part of the staple diet in Gujarat. Food and drinking water are largest sources of strontium. Strontium can enter the body through inhalation also and get deposited in lungs. Dissolved form then enter the bloodstream and a large portion gets accumulated in the bones. Strontium is mainly found in the soil. A relation between high strontium content in the bone and the distribution of calcareous rocks like limestone and chalk was established (E J Hamilton *et al*,1972). Gujarat state have large reserves of limestone and accounts for 11% of total reserves in India as per UNFC system as on 1.4.2010. Limestone is present in different varieties like marl and chalk across the state. (Indian minerals yearbook 2013, ministry of mines, Govt of India)

A relation between hard water and high strontium levels in bone was also observed. (Hamilton *et al*). Higher strontium levels in Gujarat may be attributed to the higher hardness and total dissolved salts of ground water in Gujarat. Hardness of water in Ahmedabad area range from10 to 1375mg/l during pre-monsoon season which is higher as compared to Cochin where total hardness value of ground water ranges from 41 to 536mg/l during pre-monsoon season. (status of groundwater quality in India part-2, CPCB)

Thus strontium levels deposited in body varies according to the geographical location and can be established as a geographical marker.

Strontium levels in vegetarian population of Kerala was found to be lower than the non-vegetarian population. This may be due to the increased consumption of seafood in Kerala. Sea water is a rich source of strontium and it shows the characteristic of bioaccumulation.

Strontium levels in tobacco chewers were higher as compared to the standard population who didn't use any tobacco products. Strontium is present in trace amounts in some tobacco formulations. There is no significant difference in strontium levels between tobacco smokers and standards in contrary to the increased Serum strontium levels in smokers as compared to non-smokers in a study by Bernhard *et al*.

Iron

There is a significant difference in the iron levels of Gujarat and Kerala population. Higher iron levels in Gujarat may be due to consumption of more cereals and green leafy vegetables. Out of cereals, millet, bajra and ragi are very rich source of iron. Indian rural diet derives iron mainly from cereals (77percent), pulses (8 percent) and vegetables-fruits (7 percent).(Gupta *et al* 2015) However bioavailability of iron from vegetarian diet is lesser as compared to mixed dietary habits. (Richard Hurrel 2010).

According to the central ground water board, iron levels in the ground water of Ahmedabad region is higher and is present as a contaminant. Iron levels in the ground water of Bhavnagar region in Gujarat was beyond the permissible limits in all the samples studied by Mishra *et al*, 2009.

Increased iron percentage in Gujarat may be due to the increased tobacco usage habits. Tobacco chewers showed an elevated iron levels as compared to the standards. Cigarettes, pan masala and betel quid products contains good amount of iron. EDXRF of two of the pan masala products, which is most commonly used in Gujarat showed presence of iron in 3.4% and 0.59%. Cigarette contained 1.1% of iron.

Iron present in betel quid components in µg/g (Zaidi *et al* 2002)

ARECA NUT	75± 8
BETEL LEAF	171 ±21
SLAKED LIME	190±29
CATECHU	5159±774

Sulphur

Sulphur levels in Gujarat is higher as compared to Kerala population. Within Gujarat tobacco chewers especially those who used pan masala products showed an elevated levels of sulphur in the samples. Sulphur is present as part of the formulations of the 2 pan masala products and the cigarrete analysed by EDXRF.

Sulphur is present as SO_2 in air and its levels in air determines the quality of air. The concentration of sulphur dioxide is higher in polluted cities. SO_2 levels in Ahmedabad is higher as compared to Kerala. Average Air quality index for SO_2 in Ahmedabad is 46(ranges from 24- 107). Average Air quality index for SO_2 in Kerala is 5 (ranges from 2 – 7).(National air quality index, CPCB ,Ministry of Environment, Forests and Climate change). Thus the higher sulphur levels in dental calculus of Gujarat may be due to the higher air pollution levels and the predominant habit of tobacco chewing.

Potassium

Potassium levels in Gujarat was comparatively higher than Kerala population. It may be due to the increased intake of plant based food in Gujarat. Pulses, fruits and vegetables, nuts and oilseeds are major sources of potassium. Potassium is also most abundant in soil. Mineralogy of potassium in Indian soils were reviewed by a group of scientists and they concluded that soils of north west India are abundant in potassium due to the presence of potassium feldspars and micas in the alluvial soil of these regions.(Sidhu *et al*1984). A large part of Gujarat has medium deep and deep black soil which have smectite as the dominant clay mineral and mica and kaolinite as associated minerals (G S Sekhon,1999).

Nutrient index of potassium in the agricultural soils of Gujarat was 2.60 and in Kerala it was 1.98 as estimated in 1997 (H Pathak ,2010 Indian Agricultural Research Institute).

A significant amount of potassium is lost in sweat. (Mao I F *et al* 2001, Malhotra MS *et al*, 1976). Due to the more humid climatic conditions of Kerala as compared to Gujarat, sweating is more profuse in Kerala. Annual relative humidity in Gujarat is 55% as compared to 78% of Kerala.(Indian Meteorological

Department). The loss of potassium in sweat may be the cause of lesser potassium levels in Kerala in spite of consuming potassium rich food like banana.

Thus the higher potassium levels in Gujarat may be used as a biogeomarker.

Potassium levels in people with vegetarian dietary habit was higher as compared to mixed dietary group of Kerala. This may be due to the higher intake of potassium rich vegetables and fruits like banana.

Potassium levels in tobacco users were higher as compared to standard population. A study by Wannamethee S G concluded that serum potassium levels was strongly related to smoking habit. (Wannamethee S G -1997). Potassium is present as a part of the formulations of pan masala products.(Suresh kumar *et al* 2013)

EDXRF of the pan masala products showed a significant percentage of potassium. Cigarette contained 35% of potassium. The high amount of potassium in these tobacco products and its elevated levels in the users can be used as markers for identification of these habits.

ZINC

Zinc levels in Gujarat population is higher as compared to Kerala population, in spite of the lesser consumption of zinc rich food sources like oysters,crabs, fish, red meat like beef, and poultry in Gujarat. This may be due to the higher percentage of tobacco users in Gujarat as compared to the Kerala population. Zinc levels in tobacco smokers and tobacco chewers were higher as compared to the standards. Decreased zinc levels in Kerala can also be due to the higher consumption of alcohol Approximately 30%–50% of alcoholics have low zinc status because ethanol consumption decreases intestinal absorption of zinc and increases urinary zinc excretion (National Institute of Health, Zn fact sheath) 1.6% of women and 37% of men consume alcohol in Kerala whereas only 0.3% of women and 11.1% of men consume alcohol in Gujarat. (NFHS 4- 2015-16)

Zinc levels in vegetarians were slightly lesser as compared to people on mixed diet. Zinc levels in plasma of south Indian vegatarians were found to be lesser in a study by Sreekumar *et al*.(1992)

Zinc is present as a part of the formulations of cigarettes and pan masala. Elevated zinc levels in tobacco chewers may be due to the presence of zinc in these formulations. Areca nut, pan chewing habits are more prevalent in Gujarat. Zinc is present in trace amounts in betel quid ingredients.

Zn % in different components of Betel quid – (from Zaidi *et al* , 2002)
Arcanut – 5 ± 1 µg/g
Betel leaf – 16.6 ± 2.2 µg/g
Slaked lime- 1.24 ±0.19
Catechu – 1.77± 0.27

0.8% of women and 25.7% of men in Kerala use any kind of tobacco products which is lesser as compared to Gujarat. 7.4% of women and 51.4% of men use any kind of tobacco products in Gujarat. (NFHS- 4 2015-16).

MANGANESE

Manganese was obtained from dental calculus samples of Guajarati population. 16 samples showed presence of manganese in Gujarat whereas only 1 sample in Kerala showed the presence of manganese. It was detected in dental calculus samples of patient's with tobacco usage habits.

Tobacco chewers in Gujarat who commonly used pan masala products showed presence of Manganese. Mean % of Mn in tobacco chewers was 0.174 and a mean value of .121 in tobacco smokers. No manganese was detected in standard population group.

EDXRF of 2 of the pan masala products and 1 cigarrete commonly used in Gujarat showed presence of trace amounts of manganese.

Concentration of Mn in µg/g in betel quid ingredients. (Zaidi *et al* 2002)

ARECA NUT	47± 6
BETEL LEAF	380 ±38
SLAKED LIME	57±8.6
CATECHU	170± 20

The presence of manganese in tobacco chewers can thus be used as a prospective tool in identification of these habits.

COPPER

Copper levels in Gujarat is higher as compared to Kerala. Within Gujarat tobacco chewers had higher levels as compared to standards and tobacco smokers. Copper levels in dental calculus of people who used pan masala products were higher. Copper was present in trace amounts as part of the formulations of pan masala products and cigarette on EDXRF analysis.

Copper is present as a constituent of raw areca nut, however commercially available pan products contained significantly higher copper levels (Mathew *et al* 2014). The presence of copper in areca nut and its role is oral submucus fibrosis was studied upon. (Trivedi *et al*,1997)

NICKEL (Ni)

Nickel was present in trace amounts in the dental calculus samples. There was no significant difference in the levels of Nickel between the population groups.

SILVER (Ag)

Silver is used as additives and coatings on food items. Silver was detected from 3 samples of people who used pan masala products within Gujarat population. The presence of silver as formulations in pan masala products has to be evaluated.

TIN (Sn), ZIRCONIUM (Zr) ,CHROMIUM (Cr) was detected in few samples of people with tobacco chewing habit within Gujarat.

TITANIUM

Titanium was detected from two of the samples in the mixed dietary group of Kerala. This may be due to the high presence of titanium minerals within the Kerala state. Kerala is well known for high content of Titanium enriched ilmenite deposits.(Ali *et al*). Ilmenite along the coastal sediments of Kerala is known to contain an average of up to and even

exceeding 60 wt% TiO2 (Krishnan *et al* 2001). There are many literatures on the study of Ti enriched sand deposits of Kerala beaches. (Stefan Bernstein *et al* 2007).

Exposure to titanium occurs due to its natural presence in an area in the form of ores, dietary intake, or by occupational exposures by inhalation of titanium nanoparticles.

In 1969 risk assessment of titanium dioxide (TiO2) as a food additive was carried out by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) who concluded: "Titanium dioxide is a very insoluble compound.The studies in several species, including man, show neither significant absorption nor tissue storage following ingestion of TiO2. Establishment of an acceptable daily intake for man is considered unnecessary" (JECFA 1969). However in following years many experimental studies were done for the biological effects of Ti and literatures critically reviewed the above statement.

Due to the increased use of titanium nanoparticles as food additives and in industries, it is essential to reassess the safety aspects of titanium. Occupational exposures to titanium occurs by inhalation of titanium nanoparticles. In November 2005, the United States National Institute for Occupational Safety and Health (NIOSH) proposed a recommended exposure limit (REL) for TiO2 Nanoparticles at 0.3mg/m3, which was 10 times lower than the REL for TiO2 Food particles. The International Agency for Research on Cancer (IARC) performed an assessment of TiO2 cancer potential in 2010 (IARC 2010). IARC classified TiO_2 as a human carcinogen group 2B, because there was enough evidence that nano-TiO_2 may cause lung cancer by exposure through inhalation.

Though titanium is considered as an inert metal, it is found to be present in oxide form in water bodies. The potential of TiO_2 to bioconcentrate, bioaccumulate and biomagnify in the bodies of fish has also been studied upon (Zhang *et al* 2006, Boris Jovanovic2014). TiO2 nanoparticles gets sedimented in aquatic Biosystems which are then absorbed by aquatic plants and nematodes. Floating titanium nanoparticles are attached to planktons which are then transferred to fish. The transfer of titanium oxide nanoparticles to different trophic levels were seen. These studies have indicated potential biomagnification of TiO2 via food chain transfer. (Dong Ha Nam *et al*).The toxicity of TiO_2 on fish embryo was studied by Paterson *et al*(2011).Accumulation of titanium in human bodies may result from the increased uptake of contaminated fish. There is a possibility of toxic effects in humans due to the chronic dietary intake of fish and biomagnification of titanium.

The presence of titanium from dental calculus samples of Kerala population may be due to increased dietary intake of fish. The annual per capita consumption of fish in Kerala is very high(18.5 kg) as compared to the national average (5kg) (DEPT of fisheries, govt of Kerala).. Coastal length of Kerala accounts for 590 km. Over a nearly 130 km segment of coastline from Manavalakurichi in the south, to Alleppey in the north of Kerala, ilmenite in coastal sediments, onshore and offshore, is known to contain an average of up to, and even exceeding, 60 wt.% TiO2 (Krishnan *et al*., 2001).

Trace metals in the sediments of Cochin estuaries were studied upon by Dipu *et al* (2002). Sediment samples from from 10 areas along the Cochin backwaters was collected and analysed. Results showed the presence of heavy metals like Cu, Pb, Zn,Cd, Mn,Cr, and Hg.

Ti was present in all the 10 samples in its oxide form ranging from 0.13% to 1.18%.Impact of titanium effluents on the community structure of zooplankton in the inshore waters of Lakshadweep sea at Vettukad, Thiruvananthapuram was studied upon by Wilson *et al*. Decreased incidence, density and distribution, of zooplanktons in the polluted zones revealed the adverse effect of titanium discharge on the zooplankton community in the Sea.(Wilson *et al* 2013)

Due to the widespread distribution of titanium rich ilmenite along the Kerala coasts and the presence of large scale titanium industries in Kerala, there is a high possibility of titanium bioaccumulation in Kerala population. A widespread study on the titanium levels in sea water and other inland water bodies of Kerala has to be undertaken. The potential of titanium to bio accumulate and bio magnify in the fish and reaching the higher levels of food chain has to be studied upon. Apart from the NIOSH recommended REL (Recommended exposure limit), no occupational or environmental exposure limits for titanium dioxide nanoparticles have been set by any regulatory agency. The possibility if titanium toxicity has to be considered and safety limits has to be evaluated.

Titanium can thus be established as a possible geographical marker of Kerala.

Ytterbium

Ytterbium (Yb) is a rare earth element which can slowly react with water and oxidises in air. Ytterbium was detected in trace amount from one of the dental calculus sample collected from Kerala. The sample was collected from a person residing in a coastal area, near to estuaries.

Ytterbium is most often recovered from monazite sand. Monazite contains rare earth elements and thorium. Kerala contains rich source of monazite sand. 1.90 million tonne of Monazite resources in the beach sand mineral placer deposits along the Kerala coastal area has been estimated by AMD (Atomic Minerals Directorate for Exploration and Research) – unit of DAE (DEPT of Atomic Energy).

Rare earth elements like Yb are present as sediments in aquatic ecosystem like estuaries. The role of rare earth elements as biogeochemical indicators in mangrove and coastal ecosystem has been studied upon. (Sappal *et al* 2014). In the study by Sappal *et al* sediment samples along the Pichavaram mangrove ecosystem (Tamilnadu) was analysed. 2.5±0.02 µg/g of Yb was recovered from the sample areas.Thus ytterbium can be studied upon as a probable geographical marker, especially in the identification of estuarine or coastal ecosystem.

Geographical Markers

Geographical Markers of Gujarat	Geographical Markers of Kerala
Calcium	Phosphorous
Strontium	Titanium
Iron	Ytterbium
Potassium	
Sulphur	
Zinc	
Copper	
Silver	
Zirconium	

Elevated Elements According To Lifestyle Habits

Tobacco chewing Habit	Tobacco smoking Habit	Vegetarian dietary habit	Mixed dietary habit
Manganese Strontium Potassium Iron Zinc Copper Sulphur	Manganese Zinc	Iron Sulphur	Calcium Phosphorous Strontium

CONCLUSION

It is possible to conclude that there is a significant difference in the elemental composition of dental calculus among the two population groups of Gujarat and Kerala. Geographical location, environmental conditions, dietary habits, and lifestyle habits influences the elemental composition of calculus.

It is possible to establish certain elements as geographical markers, due to their presence and elevated levels in a particular population. Calcium, Strontium, iron, sulphur, potassium, copper, zinc, silver and zirconium can be possibly established as the geographical markers of Gujarat. Phosphorous, titanium, ytterbium, can be possibly established as geographical markers of Kerala. Titanium and Ytterbium may possibly be used as geomarkers of coastal or estuarine regions.

Certain elements may be used in the identification of certain lifestyle habits like tobacco smoking, tobacco chewing, usage of certain pan masala products and also dietary habits. Certain elements like manganese, zinc, sulphur, iron, potassium, strontium and copper are seen in elevated levels in tobacco chewers, manganese and zinc levels in tobacco smokers, as compared to the people who doesn't use any tobacco products. These elements may possibly be used in the identification of these habits.

Dietary habits also play an important role in the elemental composition of dental calculus. People who follows a vegetarian dietary habit have an elevated levels of iron, sulphur and potassium as compared to people on mixed dietary habits who have an elevated levels of calcium, phosphorous, strontium and zinc.

Thus the elements in the dental calculus may be used as a prospective aid in the identification of a person based on their geographical location and lifestyle habits.

In every scientific study, there is always a limitation in the form of limited data. In this study also, there is a limitation in the form of lesser data. Though there are few elements by their presence / higher concentrations are found to be useful as geographical markers, still the data could be enlarged for a more reliable and precise results by including a larger data.

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