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Research Article

No Association was Detected between Polymorphism of COL1A2 Genes and Occurrence of Dental Fluorosis Among the Subjects Living in a Fluorosis-Endemic Area of India

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ABSTRACT

Objective: To determine any genetic association of COL1A2 polymorphism and the occurrence of dental fluorosis within an Indian human dental fluorosis population.

Material and Methods: Fifty-six (56) subjects from two groups i.e. cases with dental fluorosis from the Pavagada population (n=29) and a control group (n=27) without fluorosis, were explored. The ages ranged between 15 and 76 years (mean 50.8 years) were included, and the male to female ratio was 70:30. The severity of dental fluorosis was graded using WHO's Thylstrup-Fejerskov index (TF), and the concentration of fluoride was determined by a fluoride ion selective electrode (ISE). Genomic DNA was extracted using the standard phenol-chloroform method. The rs412777 and rs414408 polymorphism in COL1A2 were genotyped using the Sanger sequence method.

Results: Genotype distributions for rs412777 within each group were: AA 41%, AC 51%, and CC 7% for dental fluorosis participants, and AA 56%, AC 46%, and CC 0% for the control participants.

Conclusions: The rs412777 and rs414408 polymorphisms in the COL1A2gene showed no significant association between COL1A2 and the occurrence of dental fluorosis amongst this Indian population.

Introduction

Fluoride has a beneficial effect for human health when it's consumed from the drinking water – a main source, and dietary sources containing optimal concentration of fluoride or when administered via the use of oral healthcare products such as toothpastes, oral rinses, and clinically-applied varnishes, etc. in reducing the occurrence of dental caries, but a high dose may negative influence on the dental and musculoskeletal systems [1]. Dental and skeletal fluorosis is a public health concern in India since there are several fluorosis endemic areas across the country. Dental fluorosis is an irreversible tooth defect and may occur due to assimilation of higher doses of fluoride during tooth enamel development in children. Fluoride forms fluorapatite crystals and hydroxy-fluorapatite in dental enamel which can strengthen the enamel tissue to prevent demineralization, and also enhance the remineralization process [1]. The effects of fluoride causing dental and skeletal fluorosis in individual is cumulative [2, 3]. Moreover, there are two important factors that depend on the occurrence of dental fluorosis. i.e. the total fluoride intake from all sources, and the duration of fluoride exposure per person [4]. There are other causes of enamel defects which mimic dental fluorosis [5]. Epidemiological studies have found variable prevalence rates of dental fluorosis amongst different races, and this

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cannot be explained solely by the amounts of fluoride consumption and human bioregulation, including its assimilation and utilisation in the target tissues, such as teeth and bone [2, 6-8]. Fluorosis occurs in individuals who are exposed to high doses of fluoride for prolonged periods of time. However, there remains a hypothesis that fluorosis has a genetic link. Therefore, there is a need to explore the possibility of the potential role of a genetic involvement in occurrence of dental fluorosis in a population living in a fluorosis endemic area, which is prevalent in many countries including some areas of India. A recent cross-sectional study reported that the collagenase 1A2 (COL1A2) gene A/C polymorphism is involved in dental fluorosis in fluorosis-endemic areas [9]. This study included 80 children living in such an area and aimed to detect such a polymorphism in dental fluorosis cases.

However, a further study reported that the presence of polymorphism in the COL1A2 gene is not associated with dental fluorosis [9]. Environmental factors are related to development of dental fluorosis, but it needs to be elucidated whether any genetic influence or involvement for causation of dental fluorosis in fluorosis endemic areas of the affected countries. To investigate any genetic involvement a study was designed for an Indian population sampled as the population living in fluorosis endemic area of the country.

The objective of this study was to determine whether there is an association between polymorphism in COL1A2 genes and the occurrence of dental fluorosis amongst a population in India (Pavagada of the Tumkuru district of Karnataka state) where a significantly larger proportion of the population suffers from dental fluorosis.

Materials and Methods

This study was approved by the Institutional Ethics Committee of Mangalore University (Reference: MU-IHEC-2016-4), and informed consent was obtained from all the participants. The study involved analysis of the coding region of the COL1A2 gene in individuals with dental fluorosis and also in age-matched control subjects; both groups of participants resided in the fluorosis endemic area of Pavagada village of the Karnataka state of India. DNA was extracted from the blood samples using the standard phenol chloroform method. Organic (variations of phenol/chloroform) using multistep liquid chemical process that produced quality and quantity of double-stranded extracted DNA sample. Inorganic Chelex was used to cheap one-tube extraction process in which Mg2+binds to resin beads to get single- stranded DNA product, Simple extraction process in which the DNA binds to paramagnetic or silica beads was employed. The cells were lysed using gentle heat to disrupt the membranes with the assistance of SDS and DTT.

The quality and quantity of DNA was checked using 1% agarose gel, and also by spectrophotometric evaluations (50 ng/ml DNA was used for each analysis). Exons and flanking regions of the COL1A2 gene were amplified by a set of primers which were designed using the primer blast software program of NCBI. Genomic DNA was amplified in a volume of 50 ml by thermal cycling at 95˚C for 5.0 min., followed by another 35 cycles at 94˚C for 1.0 min., at a respective annealing temperature (60^0C) for 1.0 min., and then at 72˚C for 1.0 min. The exons rs412777 and rs414408 of the COL1A2 gene were sequenced for both groups using a 3730 XL DNA analyser (Applied Biosystems). Sequences obtained were analysed and compared with respective reference sequences obtained from GenBank via auto assembler software.

Statistical Analysis

To elucidate the genetic association of rs414408and rs412777 with dental fluorosis, the alleles were counted, and Chi-squared contingency table analysis was carried out using Plink software. The Hardy-Weinberg equilibrium (H-WE) test was performed in control groups using Plink software (p values was set at < 0.05 for this study) as well.

Table 1: Distribution of *COL1A2* genotypes and allelic frequencies in the fluorosis groups and control groups.

CHR	SNP	Position	Mutant Allele	Frequency in cases	Frequency in controls	Wild type	P value	Odds ratio
7	rs412777	94412625	C	0.2222	0.3276	A	0.2908	0.5865

Table 2: Association analysis of rs412777 with dental fluorosis in Pavagada population of South India.

	Fluorosis group (n=29)	Control (n=27)	Total (n=56)
Genotype rs412777			
AA	12 (0.413)	15 (0.56)	27 (0.482)
AC	15 (0.512)	12 (0.46)	27 (0.482)
CC	02 (0.068)	0	02 (0.035)
Genotype rs414408			
AA	0	0	0
AC	0	0	0
CC	29 (1.00)	27 (1.00)	56 (1.00)
Allelic frequencies for rs412777			
A	0.67	0.78	
C	0.33	0.22	
Allelic frequencies for rs414408			
A	0	0	
C	1	1	

Results

Both SNP's (rs414408 and rs412777) were found to be in H-WE (p = 1.00 and 0.67 respectively). This suggests that samples were collected randomly, and also the genotyping error was not present in the datasets. The association analysis of rs414408 was excluded since the mutant allele was not observed in the dental fluorosis group, also in control group (Table 1). The genotype distributions for rs412777 and rs 4144o8 in both the groups are shown in (Table 2). Using the rs412777 marker, the allele frequencies of rs412777 and rs414408 of the COL1A2 genotypes were counted as 67% and 33% for the wild-type and mutant alleles respectively for the dental fluorosis group, and 78% and 22% respectively for the control group. However, the rs414408 marker mutant allele was not observed in both the groups. rs412777 was not expressed significantly to see an association of the gene for causation of dental fluorosis in this cohort (p = 0.29).

Discussion

The study shows a preliminary finding amongst a sampled population of the Pavagada of Tumkuru district of the Indian state of Karnataka, which was statically sampled from a highly prevalent (95%) rate of dental fluorosis. This study aimed to investigate whether a specific gene is responsible for the causation of dental fluorosis or not. Although few researches reported a strong correlation of the COL1A2 gene with the occurrence of this condition, the current study among an Indian fluorosis endemic area does not show any genetic association [10-13]. Other studies [11, 14] revealed that this gene polymorphism could be a rare condition. On the contrary a study explained that a gene polymorphism is rare in dental fluorosis [15].

However, the influence of an explicit candidate-gene may have a relationship between expression of polymorphisms in the COL1A2 gene and causation of dental fluorosis among Chinese children with dental fluorosis [11]. Also a case control study conducted by among 8 -2 years old children suffered from dental fluorosis (n = 75) and without dental fluorosis (n = 1650), found that there was increased risk of dental fluorosis compared to the children carrying the same genotype (pp) in a fluorosis endemic area compared to control in the same locality [11]. Hence, the findings showed an association between polymorphisms in the COL1A2 gene and the occurrence of dental fluorosis in a population exposed to high fluoride levels in drinking water [11]. Another study in Brazil revealed a strong heritability connection in development of dental fluorosis, and the study conducted among 384 twin pairs revealed that, 95% of fluorosis is attributable to genetic factors [12, 14].

Moreover, an animal study in mice found an association of dental fluorosis to and gene, the study designed based on age, gender, food and drinking water (estimating the fluoride concentration), the study also aimed to investigate correlations between fluoride concentrations in mice mottled teeth with variable severity of dental fluorosis and genetic influence [16]. However, the genetic association in dental fluorosis in any severity in mice teeth was inconclusive. Similarly, Vieira *et al.* (2005) studied 72 male mice, using the same three strains of mice as those of Everett *et al.* (2002), find no genetic involvement in dental fluorosis [5, 16].

Mousny *et al.* (2006) found that the genetic influence on fluorosis was inconclusive [17]. Therefore, several researchers studied with three mice strains, and demonstrated different level enamel fluorosis. A study by Everett *et al.* (2002) suggested that genetic factors may contribute to a varied level of bone response which may affect bone density, needs to be invested extensively [16]. Yan *et al.* (2007) found constructive metabolism in B6 and enhanced osteoclast genesis shifts in hematopoietic cell differentiation in the C3H strain, may have an influence, needs further investigation [18]. However, from the published researches, we conclude that fluoride may exert a varied impact on bone density, and that this affect enamel, and that may be species-dependent, possibly genetically determined, but in human it is not conclusive.

Another en-depth study carried out by Zhang *et al.* (2006), showed that a concentration of only 10 µmol. /L sodium fluoride decreases the quantity of a cellular component and suppressing the expression of matrix metalloproteinase-20 in human fetal tooth ameloblast lineage cells, may influence dental fluorosis [19]. But he added that, the concentration of sodium fluoride did not alter the amount of amelogenin or kalikrein-4 (KLK-4). Mechanism-wise during ameloblasts, fluoride may alter the cytoskeleton through interference with the Rho signalling pathway. This was also demonstrated by Li *et al.* (2005) by using murine ameloblasts, and that was demonstrated the cellular response to fluoride include an elevation of F-actin in ameloblasts and results in RHO/ROCK pathway [20]. The study also reported on the mechanism involved in fluoride's alteration of amelogenesis and dentinogenesis processes. In turn, these fluoride-modified processes can modify fluoride's actions on mineralized tissue [11].

The molecular mechanisms of the influence of COL1A1 and COL1A2 on bones and teeth is at least partially explicable by the following findings: 1) the COL1A2 gene provides the code for the biosynthesis of type I collagen, and collagens represent a family of proteins that strengthen and support many tissues, including cartilage, bone, tendons, tooth tissue, skin, and the eye the sclera (type I collagen is the most abundant form of collagen in the human body); 2) two genes, COL1A1 and COL1A2 (collagen type I-α-1 and collagen type 1- α-2), are responsible for collagen formation. Specifically, the COL1A1 gene produces a component of collagen type I known as pro-α-1 chain, whilst the COL1A2 gene produces the corresponding pro-α-2 chain [18, 21].

These chains combine to synthesise type I procollagen, and collagenase enzyme is a fibril-forming collagen found in the majority of connective tissues and is abundant in the bones, cornea, dermis, and tendons. However, collagen fibres in hard tissue, such as those in bones and teeth, are mineralized by calcium hydroxyapatite, and there is therefore an enhancing role for safer doses of fluoride. Therefore, further investigations involving larger samples size are required to address these questions.

Conclusions

There was no role for the gene COL1A2 in the occurrence of dental fluorosis amongst the Pavagada population in India. Further studies are required in order to establish if rs412777 acts as a genetic risk factor for dental fluorosis.

Conflicts of Interest

None.

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Author Contributions

Chitta Ranjan Chowdhury: Designed the research, guided, coordinated and confirmed the final draft; Shahnawaz Khijmatgar: Collecting samples, literature search and drafting; Mohammed S Mustak: Guided laboratory investigation related to gene; Avidyuti Chowdhury: Critical analysis and editing; Divya Kumari P: Supported with Fluoride estimation; Edward Lynch: Critical analysis and editing; Martin Gootveld: Statistical input, critical analysis and editing.

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