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ASSOCIATION OF XEDAR RS 372024 GENE POLYMORPHISMS IN SKELETAL CLASS I CASES WITH CROWDING AND INCREASED TOOTH SIZE IN SOUTH INDIAN POPULATION USING DNA SEQUENCING

KEYWORDS

Crowding, Class I malocclusion, XEDAR Gene Variant rs 372024, salivary diagnostics.

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ABSTRACT Dental crowding is a problem for adolescents and adults in modern society and is a complex anomaly affecting esthetics and quality of life.39 The objectives of study were to evaluate genetic polymorphisms in XEDAR gene variant rs 372024, in skeletal Class I crowding cases. The saliva samples were collected from 60 subjects; 30 individuals had Class I skeletal jaw relationship with crowding, while the controls included 30 subjects with skeletal Class I profile. DNA was extracted from the saliva samples and subjected to polymerase chain reaction. After amplification of the selected fragments, DNA sequencing was carried out. The results were obtained as electropherograms. This study indicates that there is no association between the presences of XEDAR gene variant rs 372024 with the incidence of dental crowding in our population.

INTRODUCTION

The development of dentition is a fascinating process that encompasses a series of epithelial–mesenchymal interactions involving growth factors, transcription factors, signal receptors and other morphogens. It is not surprising that such a complex process is prone to disturbances.

Crowding is the lack of space for all the teeth to fit normally within the jaws. The teeth may be rotated or displaced. Crowding occurs when there is disharmony in the tooth-to-jaw size relationship or when the teeth are larger than the available space.[2]

Genetic polymorphisms have been successfully reported for several forms of dental anomalies including Amelogenesis Imperfecta and hypodontia.11 EDA and BMP2 genes are considered responsible for syndromes like Ectodermal Dysplasia affecting teeth as well.11A new approach for sequencing the DNA is the Automated DNA sequencing procedure where each nucleotide was labelled with fluorescent dyes.

Advances in genetic testing, gene therapy, pharmacogenomics, mechanogenomics, and stem cell therapy are likely to produce the most dramatic changes in orthodontic treatment. Blood specimens are used extensively to monitor the general state of health and for analysis of many specific diagnostic analytes. Non-invasive technology has thus become increasingly important and would be ideal for point-of-care diagnosis.[12]

EDA gene is located on long arm of the X chromosome between positions Xq12-q13.1 (region 1, band 2 and region 1, band 3 sub band 1).11 Mutations of this gene can lead to Ectodermal Dysplasia involving anomalies of development of ectodermal organs which include sweat glands, hair follicles and teeth. The EDA gene mutations could also result in X linked hydrotic form of ectodermal dyspalsia and it causes a selective type of tooth agenesis which is syndromic. Mutation of human EDA gene could potentially lead to differential gene expression that causes large tooth phenotype, which has been suggested to cause dental crowding.[11]

Therefore, analysing the association of the XEDAR gene with dental crowding, can give an in-depth knowledge, regarding the genetic influences on incidence of crowding. Hence, this study was conducted to analyse the association of genetic polymorphisms in XEDAR gene variants rs 372024 among Skeletal Class I crowding cases.

OBJECTIVES OF THE STUDY

1. To evaluate the association of genetic polymorphisms in XEDAR gene variant rs 372024 in Skeletal Class I crowding cases.
2. To evaluate the association of XEDAR gene in increase in tooth size in skeletal class I cases.

MATERIALS AND METHODOLOGY

The present study aims at investigating the association of XEDAR gene variant rs 372024 in skeletal Class I crowding cases. The polymorphism in XEDAR gene variant rs 372024

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were detected using the Polymerase Chain Reaction (PCR) test followed by DNA Sequencing. Automated DNA sequencing procedure was selected for the sequencing of DNA where each nucleotide was labelled with fluorescent dyes. Thus, when the DNA fragments were placed on the electrophoresis gel and passed through a laser beam.

OUTLINE OF THE STUDY:

60 Subjects

30 cases (GROUP A) 30 controls (GROUP B)

STEP -1 Saliva Sample (5ml)

STEP-2 Purification of salivary sample

STEP-3 Isolation of Genomic DNA
(XEDAR gene rs 372024)

STEP-4 Polymer Chain Reaction

STEP-5 DNA sequ :ing (ABI machine)

Re 'ts

Statistical Analysis

Salivary samples from 60 subjects, who visited Department of Orthodontics and Dentofacial Orthopaedics, D.A.P.M.R.V. Dental College, Bangalore, were taken after written informed consent (annexure I and II).

These were divided into two groups:
Group A: 30 subjects with Skeletal Class I crowding
Group B: 30 Controls with Skeletal Class I profile without visible crowding or spacing (+/-2mm).

Inclusion criteria for Group-A subjects:
1. Healthy and no previous history of orthodontic treatment.
2. Full permanent dentition and age less than 27 years
3. Diagnosed with a Skeletal Class I relation according to ANB angle (00 - 40), Witt's appraisal (0 to -3mm), and pleasant facial profile
4. Not less than 5mm of crowding in at least 1 arch

Exclusion criteria for Group-A subjects:
1) Class II molar relation or Class II jaw relation
2) Class III molar relation or Class III jaw relation
3) Congenitally missing teeth or missing teeth due to caries
4) Retained deciduous teeth
5) Spacing of more than 2mm
6) Supernumerary teeth

The method was divided into five steps:
Step 1: Collection and storage of saliva samples,
Step 2: Purification of salivary sample
Step 3: Isolation of Genomic DNA,
Step 4: Polymerase Chain Reaction Test (PCR),

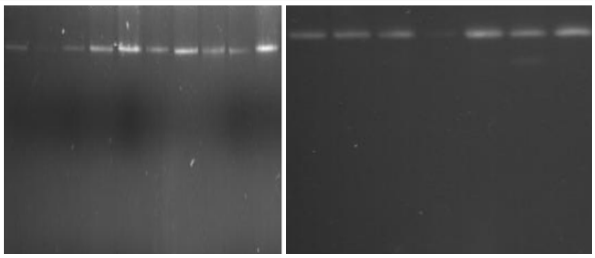

Fig 1:- Column purification image

Gene	PCRProduct Size
EDA2R	250bp

LEDA2RFP:5'GTGCCACCCAGCAAGGAGAC-3'
EDA2RRP:5'CCAAGGTCTCAGCTCCAGTAT-3'

	Mastermix	
Components	1X	17X
D.D.H20	21ul	357
2X PCR Master MIX	25 µl	425
*Forward Primer	1µl	17
*Reverse Primer	1µl	17
Template	2ul	34
Total Volume	50 µl	850

48µl of Mastermix was aliquoted into 17 different labeled PCR vials with respective primers and to this 2 µl of different template DNA was added. The PCR was set.

Cycle conditions:

Temperature	Time	No. of cycles
94˚C	5 minute	1
94˚C	30 second	30
58˚C	30 second	
72˚C	30 second	
72˚C	7 minutes	1

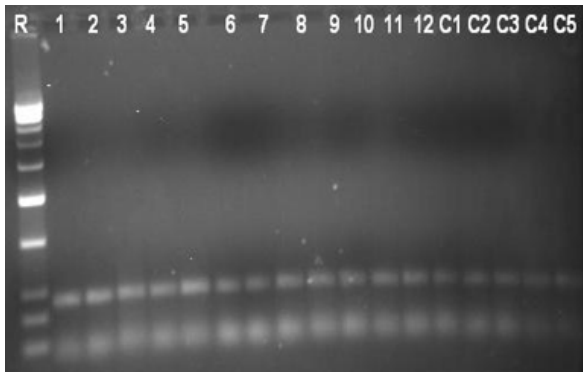

Fig 2 :- Amplified products were gel purified and the samples were sent for sequencing .

The Polymerase chain reaction (PCR) is an in vitro technique which allows the amplification of a specific deoxyribonucleic acid (DNA).

Primers used in the study for EDA gene (rs372024):
F: 5'GTGCCACCCAGCAAGGAGAC'3
R: 5'CCAAGGTCTCAGCTCCAGTAT'3

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This reaction mixture in the PCR tubes was placed in the PCR machine which was set programmed to repeat the following three staged cycle 35 times.

Stage1: Separation/Denaturation: The double stranded DNA is denatured by heat, by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA templates at 95 °C for 3 min, followed by actual denaturation at 95 °C for 1 min

Stage 2: Priming/Annealing: The primers anneal to the single-stranded DNA template at 58 °C for 1 min.

Stage 3: Polymerization/Elongation: The DNA polymerase recognizes the primer and makes a complimentary copy of the template at 72 °C for 1 min. DNA sequencing was performed using Frederick Sanger's dideoxy sequencing method in an automated ABI sequencer machine based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication.

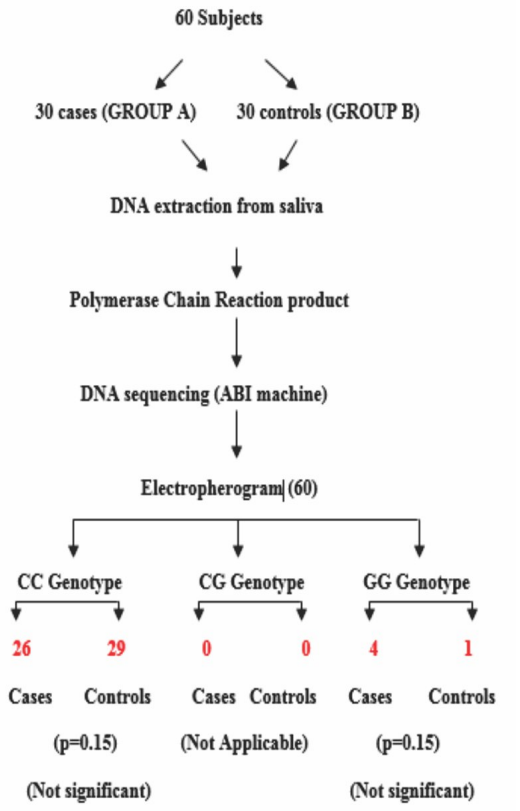
RESULTS

In the study, relationship between XEDAR gene variant rs 372024, among Skeletal Class I crowding cases was evaluated in 60 subjects consisting of Group A (P1-P30) with crowding and Group B (C1-C30) without dental crowding using Polymerase Chain Reaction (PCR) test followed by DNA sequencing.

Results for XEDAR gene rs 372024:

For rs 372024 three genotypes can be possible:

C/C	**Normal homozygous allele**
C/G	**Mutant heterozygous allele**
G/G	**Mutant homozygous allele**



Flowchart 5: Results of EDA gene variant rs 372024

After statistical analysis (Z- test):

The test results revealed CC (Non-Mutant Homozygous) genotype showed a higher proportion of 96.7% in controls as compared to cases with 86.7% , which yielded a difference of 10% (95% CI , 4%-24%)

However, this difference in proportion of 10% between cases and control with respect to CC (Non-Mutant Homozygous) genotype was not statistically significant, p=0.15

The test also revealed GG (Mutant Homozygous) genotype showed a higher proportion of 13.3% in cases compared to controls with 3.3% , which yielded a difference of 10% (95% CI , 4%-24%)

The difference in proportion of 10% between cases and control with respect to GG (Mutant Homozygous) genotype was not statistically significant, p=0.15

The association between CG (Mutant Homozygous) genotype in cases and controls was not applicable.

CONCLUSION

The conclusions drawn from this study are:-

1. The percentage of GG genotype (mutant homozygous) expression was higher in cases by 13.3% compared to controls which had 3.3% chance of mutation and the presence of CC genotype (non-mutant homozygous) was higher in controls by 96.7% compared to cases which had 86.7% chance.

2. This study indicates that there is no association between the presences of XEDAR gene variant rs 372024 with the incidence of dental crowding in our population.

3. This study also emphasize on using non-invasive techniques for genetic studies using saliva, which yields similar results when obtained using blood and it is more patient beneficial.

4. A Meta-analysis in a wider range of patient groups with more complete clinical descriptions of affected individuals may be required to more completely characterize the association between genetic markers and dental crowding.

This is in contrary to the study done by T Y Ting, Ricky Wing Kit Wong, and A. Bakr M. Rabie in Hong Kong Chinese population.11 Contradictory results are probably due to genetic heterogeneity, incomplete penetrance, limited sample sizes and different study designs, hence the null hypothesis is accepted indicating there is no association of XEDAR gene variant rs 372024 gene polymorphism in Skeletal class I cases with crowding in our population.

Studies targeting a large sample size and a number of genes are required for a better insight and understanding of the complexity of genetics. A Meta-analysis in a wider range of patient groups with more complete clinical descriptions of affected individuals may be required to more precisely characterize the association between genetic markers and dental crowding.

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