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Role of Single Nucleotide Polymorphism of Matrix Metalloproteinase (MMP-13 rs2252070) in the Etiology of Dental Caries: A PCR Based Study

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Abstract

Title: Role Of Single Nucleotide Polymorphism Of Matrix Metalloproteinase (MMP-13 rs2252070) In The Etiology Of Dental Caries: A PCR Based Study.

Background: Dental caries is multifactorial in origin. This study provides an alternative hypothesis for causation of dental caries and emphasize that genetics has a major role in disease process.

Methods: The subjects were divided into 4 groups based on oral hygiene and dental caries experience. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) was used in this study. Blood samples (2 ml) were obtained from the patients followed by isolation of Genomic DNA, Polymerase chain reaction test and digestion with enzyme Bsr-I.

Results: The size of the initial PCR products was 450bp. After digestion with restriction enzyme Bsr-I the 450bp products were completely digested with two restriction sites (250 &180) in a group of subjects with good oral hygiene and presence of dental caries. Other groups had incomplete digestion With Bsr-I.

Conclusion: Due to the single nucleotide polymorphism of MMP-13(rs2252070) the patients were suffering from dental caries despite having good oral hygiene. Genes play a major role in progression of dental caries.

Practical implication: The established relation will help in formulating guidelines for prevention and treatment of cariogenic subjects more effectively. The pattern of dental caries can be learned in more precise manner.

Keywords: Dental caries, Matrixmetalloprteinase-13, Single nucleotide polymorphism

1. Introduction

Dental Caries is an infectious microbiologic disease of the teeth that results in localized dissolution of the inorganic constituents and destruction of the organic structures. It is one of the most common chronic diseases of modern times affecting more than 80% of the general population not confined to any particular age or group.[1]

The occurrence and progression of caries is known to be influenced by numerous environmental factors, including microbial flora, salivary flow and exposure to fluorides. However, these variables alone may not entirely explain the disease development.[2] There is evidence emerging showing genetic component susceptibility and studies in human that are concluding that variation in enamel formation genes may contribute to caries development. Genes related to enamel development and mineralization such as amelogenin, ameloblastin, tuftelin and enamelysin have been associated with caries.[3] Host response to bacterial colonization is also affected by individual genetic background. Functional polymorphisms in the Matrixmetalloproteinase (MMP) genes have been attributed to enamel development and caries pathogenesis.[4] MMP has a major role in the organization of enamel and dentin organic matrix formation and may regulate mineralization by controlling the proteoglycan turnover suggestive of either control or progression of caries.[5] Various studies on the expression of MMPs have been carried out in different groups of population. [3-6]

In this study, we propose to demonstrate the role of a single nucleotide Polymorphism of MMP-13(rs2252070) in the etiology of dental caries in patients visiting the Department of Conservative Dentistry and Endodontics at College of Dental Surgery, B.P. Koirala Institute of Health Sciences, Dharan, Sunsari, Nepal.

2. Methodology

The study was ethically cleared from B.P.Koirala Institute of Health Sciences Institute Ethical Board(IRB) and written consent was taken from every patient before starting the study which was approved by the ethics committee. The study has been conducted in full accordance with the World Medical Association Declaration of Helsinki.

2.1 Sampling Technique

A non-probability based purposive sampling was used in the study. A total of 60 samples were taken in the study (15 samples in each group) within the constraints of the inclusion and exclusion criteria.

The study had four groups

Group 1: Subjects with poor oral hygiene and absence of dental caries.

Group 2: Subjects with poor oral hygiene and presence of dental caries.

Group 3: Subjected with good oral hygiene and absence of dental caries.

Group 4: Subjects with good oral hygiene and presence of dental caries

2.2 Inclusion Criteria [12]

The age of the patient ranging from 15-30 years.

The DMFT score $\geqslant$1 (for group 2 and group 4)

The DMFT score =0 (for group 1 and group 3)

2.3 Exclusion criteria [12]

Patient with history of any past medical disease (Hereditary fructose intolerance, Primary immunodeficiency, Congenital chloride diarrhea, Growth hormone deficiency, Down syndrome, Turner syndrome).

2.4 Assessment of Oral hygiene Status

Oral hygiene status was accessed by simplified Oral hygiene Index (OHI-S). The interpretations for good and poor oral hygiene were made on the basis of obtained scores:

Good: 0.0 to 0.12

Fair :0.13 to 3.0

Poor: 3.1 to 6.0

2.5 Determination of Caries Experience

Subjects were clinically examined with a probe and mouth mirror. The caries were assessed according to the criteria recommended by the World Health Organization guidelines using the DMFT.

2.6 Method of collection of Data

The methods for collection of data were divided into four steps:

Step 1: Collection and storage of blood samples

Step 2: Isolation of Genomic DNA and characterization

Step 3: Polymerase Chain Reaction Test

Step 4: Digestion with Restriction Enzyme Bsr-I.

Explanation for every step is done in details

Step 1: Collection and storage of blood samples

Collection of blood (2ml) in EDTA coated tubes and transported to the laboratory. The Storage of blood samples were done at -20 ° C. The collected blood was centrifuged and buffy coat was obtained. The obtained Buffy coat was used for isolation of DNA.

Step 2: Isolation of genomic DNA

Isolation of Genomic DNA (phenol Choloform method)

↓

Microcentrifugetubes: Blood sample (100µl) + Tris HCL buffer solution (400µl) +

↓

Proteinase K (10mg/ml) + Sodium Dodecyl Sulphate (SDS 10%): (Detergent for cell lysis)

↓

Incubation at 37 ºC for 30 min

↓

Phenol treatment to remove proteins

↓

Chloroform treatment to remove Phenol

↓

Ethanol (100%) treatment to concentrate and precipitate the DNA

↓

Ultracentrifugation at 10K for 5 min. at 37 ºC

↓

Genomic DNA Precipitat

↓

Characterization and Quantification (Optical density at 260nm and 280nm)

↓

Pure DNA Optical density (OD) = A260/A280 ⩾ 1.8

↓

Step 3: Polymerase chain reaction test

Genomic DNA precipitates (50-100ng/µl)

↓

PCR tube: DNA precipitate (2.0µl) + Taq polymerase (1.0µl)(DNA polymerase enzyme)

+ Tris HCL buffer solution (20.0µl)(to maintain pH) + PCR Primers(250mmol/l) (Forward) 50 -GATACGTTCTTACAGAAGGC-30

(Reverse) 50 -GACAAAT-CATCTTCATCACC-30

+ Distilled water (rest volume till 20.0µl) (reaction medium)

↓

The PCR amplification protocol consisted of on an initial denaturation at 94.8° C for 10 minutes, 32 cycles of denaturation at 94.8 ° C for 30 seconds, annealing at 54.8 ° C for 30 seconds, extension at 72.8 ° C for 30 seconds, and a final extension at 72.8 ° C for 5 minutes

↓

PCR Products of MMP13

↓

Gel electrophoresis (2% agarose gel with ethium bromide) for separation of base pairs

↓

UV transilluminator and Gel documentation Centre: Specific bands of base pairs of amplified PCR products

↓

Amplicon size (bp=450)

Step 4: Digestion with restriction enzymes

Amplified PCR Products (450 bp)

↓

PCR tubes: PCR products (15 µl) + Tris HCL buffer solution (3 µl) + restriction enzymes+ distilled water (1 µl)

↓

Digestion of PCR products with Bsr-I

↓

Digested PCR Products subjected to GEL ELECTROPHORESIS (2% agarose gel with ethium bromide) for separation of base pairs

↓

UV transilluminator and Gel documentation Centre: Specific bands of base pairs of digested PCR products. The 450 bp PCR product then will be cleaved into two fragments of 250 and 180 bp

only in the presence of the MMP13 – 77 G allele, leaving the remaining the 450 bp fragment un cleaved in the presence of the MMP13 – 77 A allele.

2.7 Demographic Profile of the Participants

Table 1: Distribution of subjects on basis of age

Mean Age Group	Group I	Group II	Group III	Group IV
	22.26	21.5	20.5	20.2

Table 2: Distribution of patients on the basis of gender

Group	Male	Female	Total	Chi-square	P (Value)
I	7	8	15	1.61	0.657
II	6	9	15		
III	9	6	15		
IV	6	9	15		

2.8 Statistical Analysis

Chi-square test was used to find the significance of association of MMP-13 (rs 2252070) with gender (table 2) at 95% confidence (p = 0.05).

$$X2= \frac{(O-E)^2}{E}$$

The value of Chi-square with respect to gender was 1.61 with P value of 0.65(Table 2)

3. Results

3.1 Results of the Polymerase Chain Reaction Test

The initial PCR product of the MMP-13 gene were obtained for 60 subjects which were grouped into 7 column A (1-12), B (13-19), C (20-26) and D (27-38), E (39-45), F(46-52)and G(53-60).The size of this PCR product was 450bp.(Figure 1 -7).

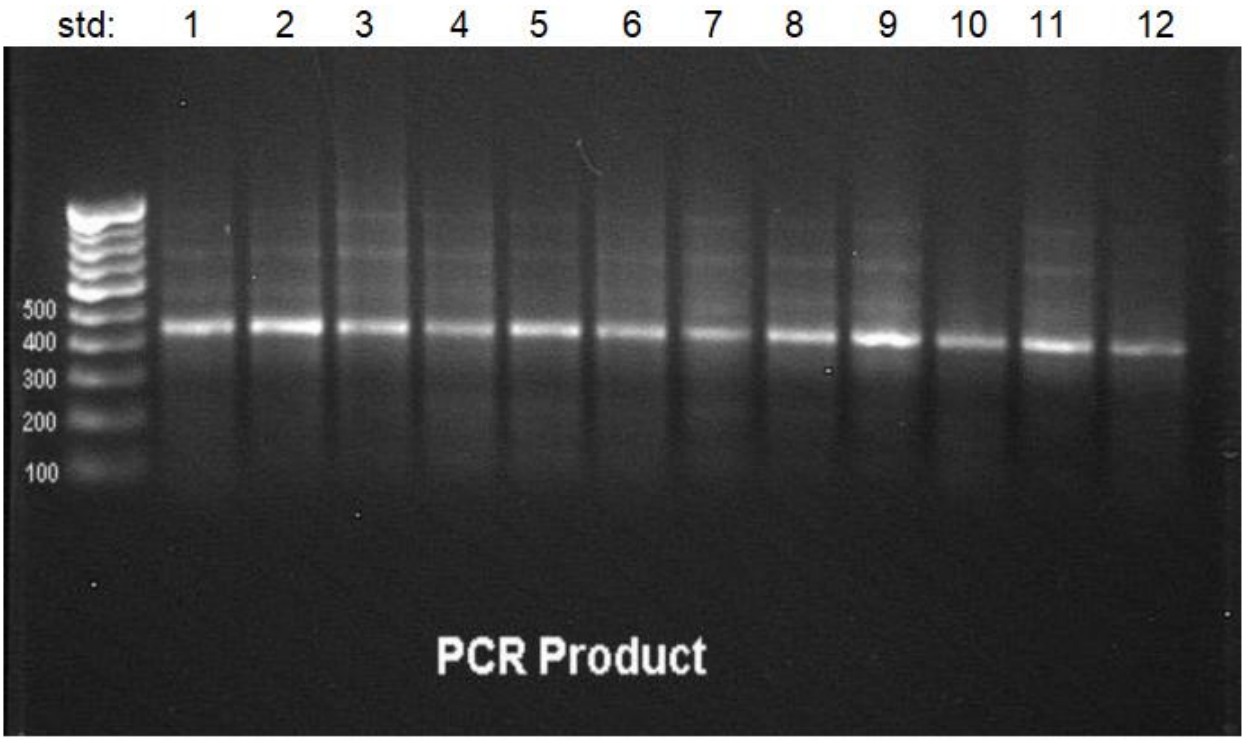

Figure 1 (Column A): Initial PCR products for 12 patients. The size of this PCR product was 450bp

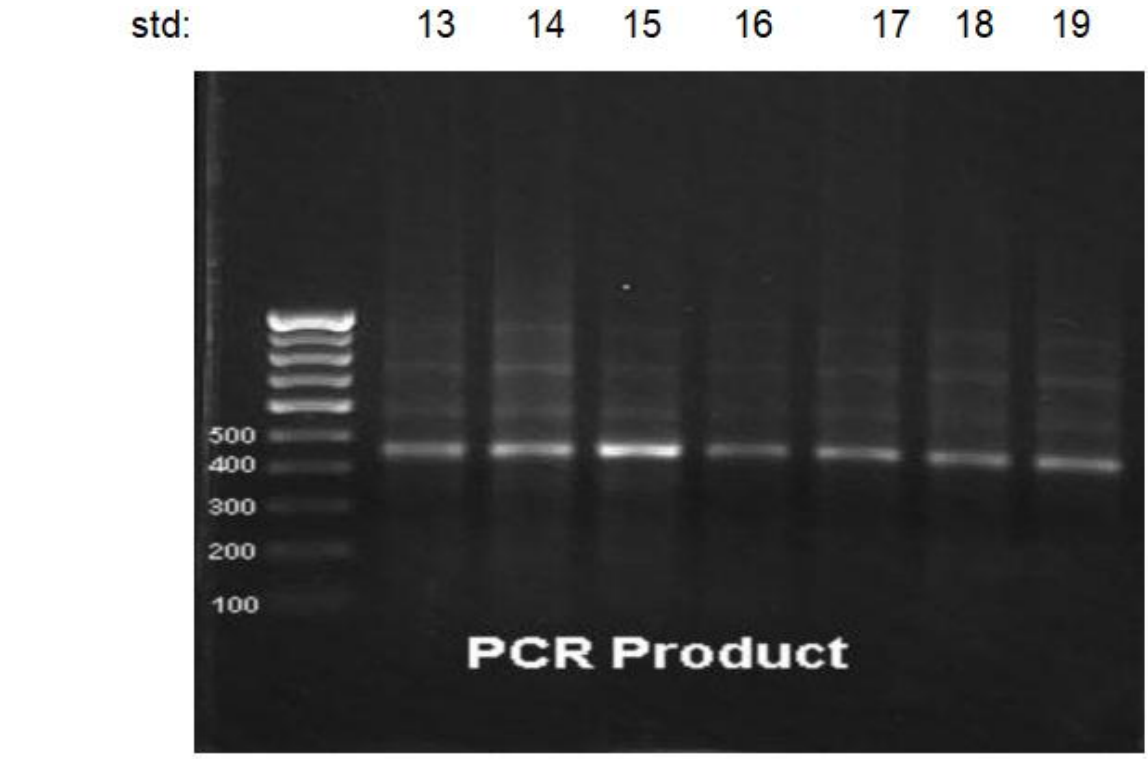

Figure 2 (Column B): Initial PCR products for 7 patients. The size of this PCR product was 450bp

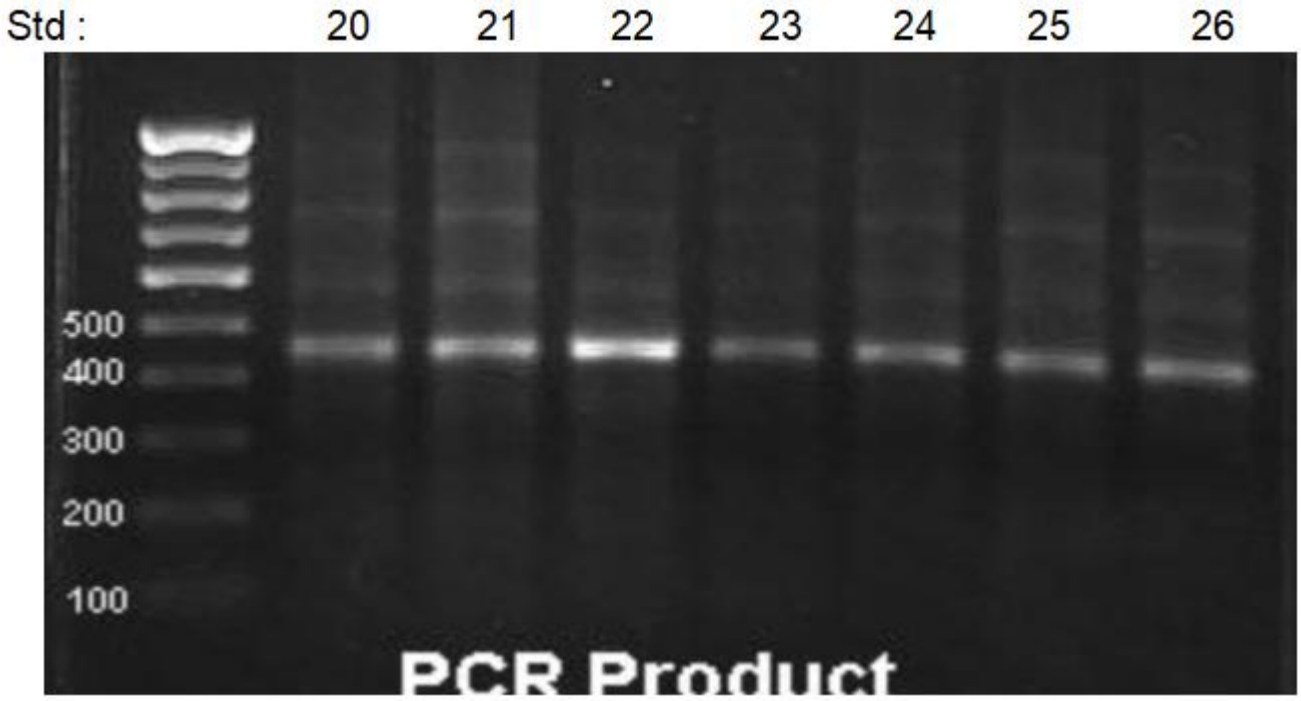

Figure 3 (Column C): Initial PCR products for 7 patients. The size of this PCR product was 450bp

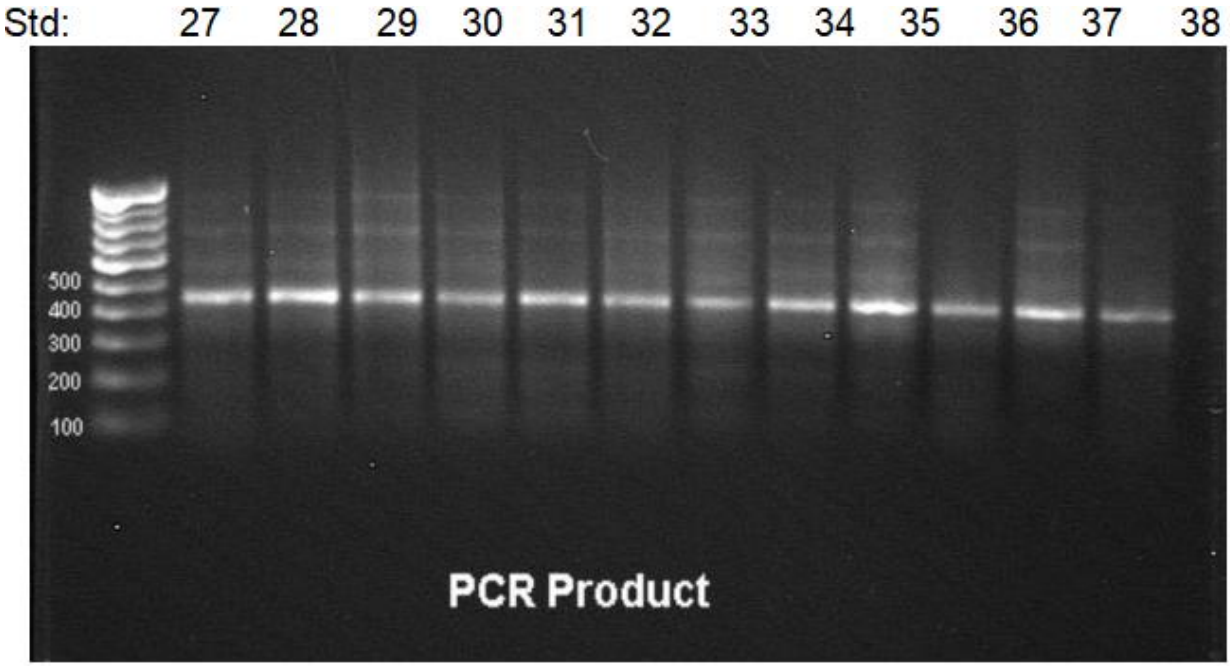

Figure 4 (Column D): Initial PCR products for 12 patients. The size of this PCR product was 450bp

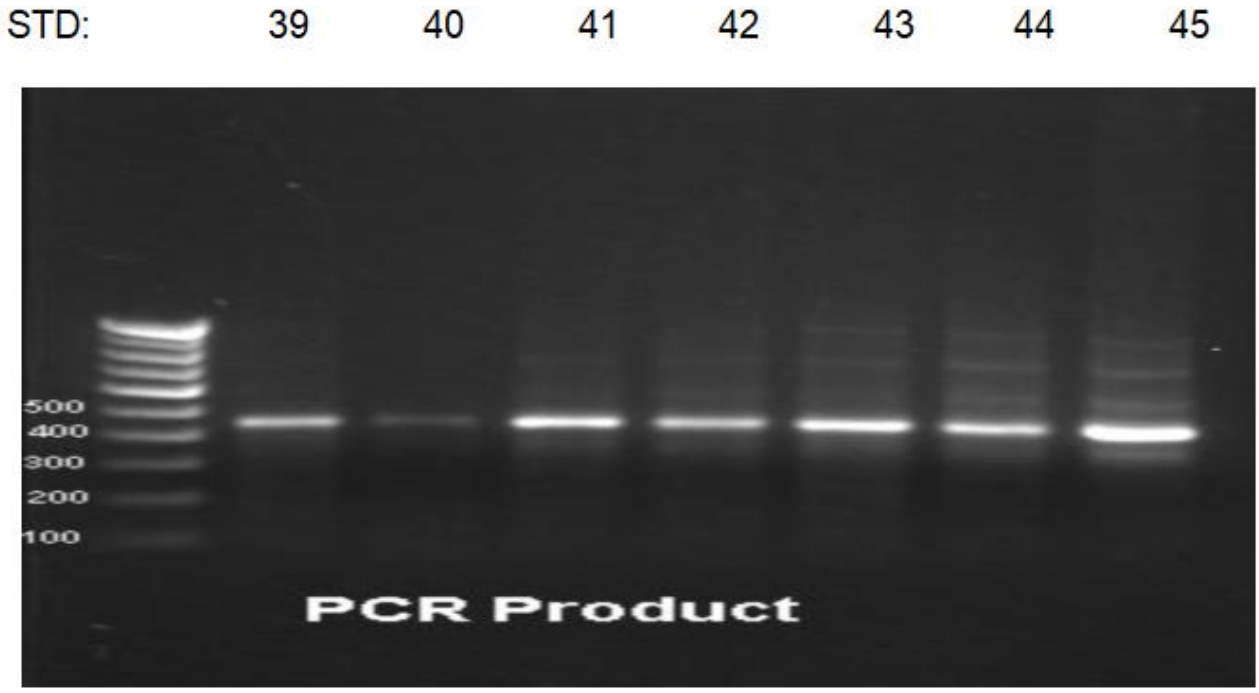

Figure 5 (Column E): Initial PCR products for 7 patients. The size of this PCR product was 450bp

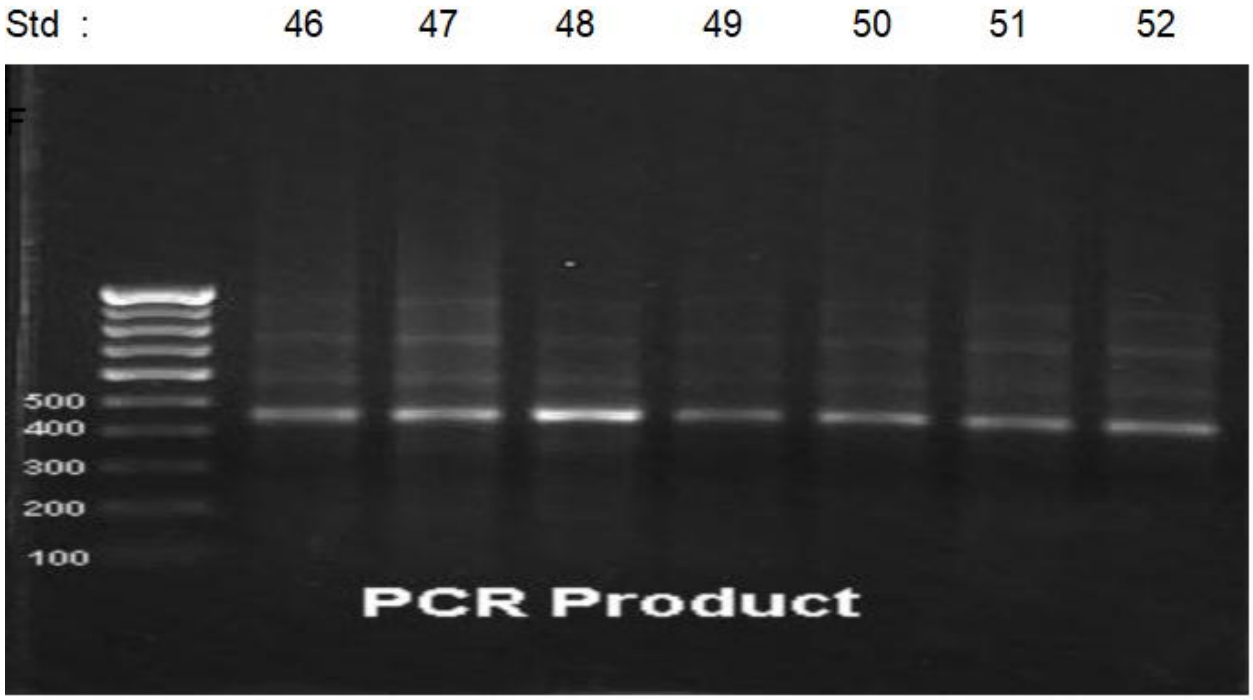

Figure 6 (Column F): Initial PCR products for F patients. The size of this PCR product was 450bp

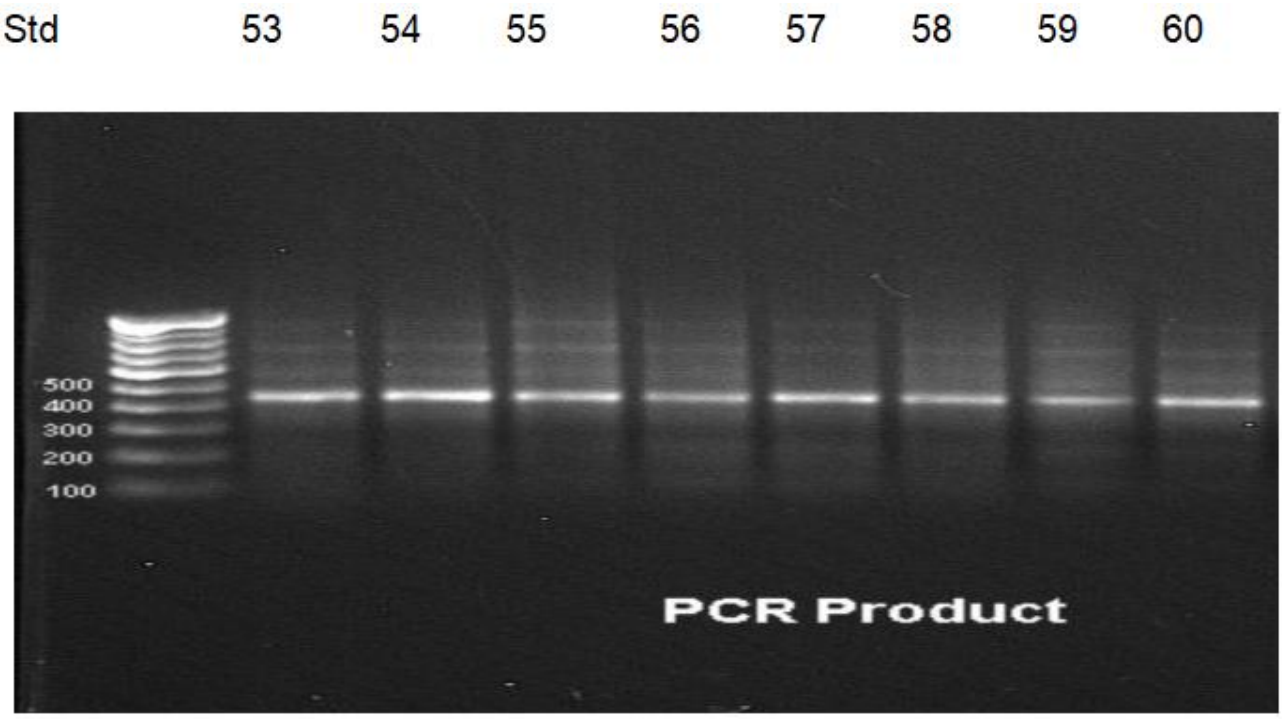

Figure 7 (Column G): Initial PCR products for 8 patients. The size of this PCR product was 450bp

3.2 Results after digestion with specific enzyme Bsr-I

After obtaining the initial PCR products of MMP-13gene (450bp)from column A to G, samples were then subjected to digestion with the specific restriction enzyme Bsr-I for groups all group (fig 8-fig11).

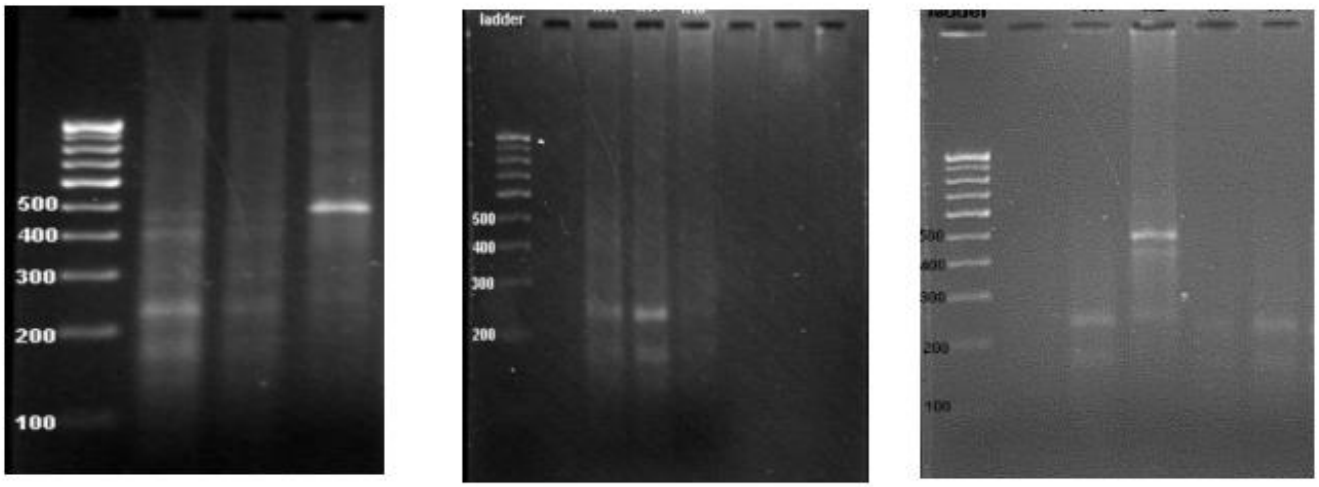

Figure 8: Group A (poor oral Hygiene and absence of dental caries showing incomplete or absence of Digestion)

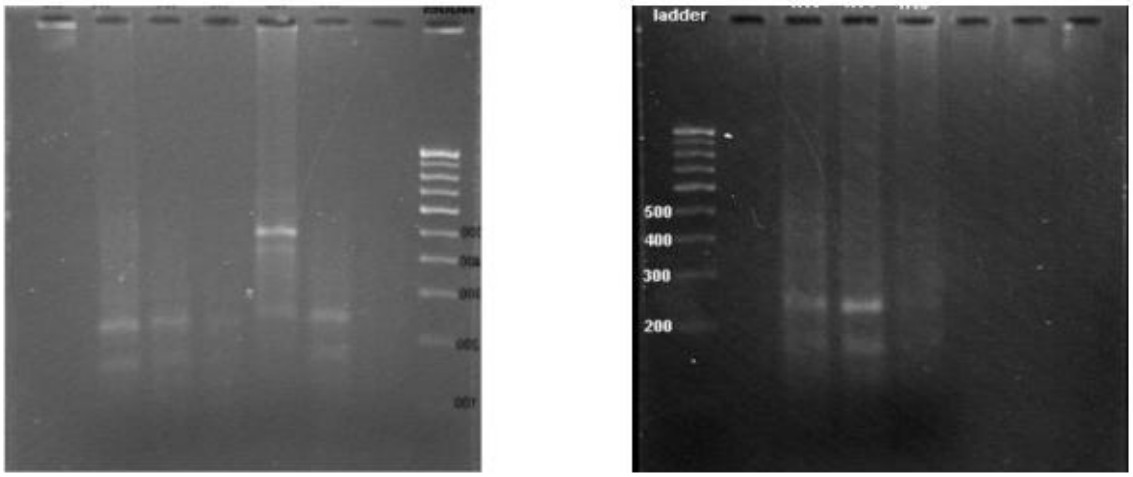

Figure 9: Group B (Poor hygiene and presence of dental caries showing incomplete or absence of digestion)

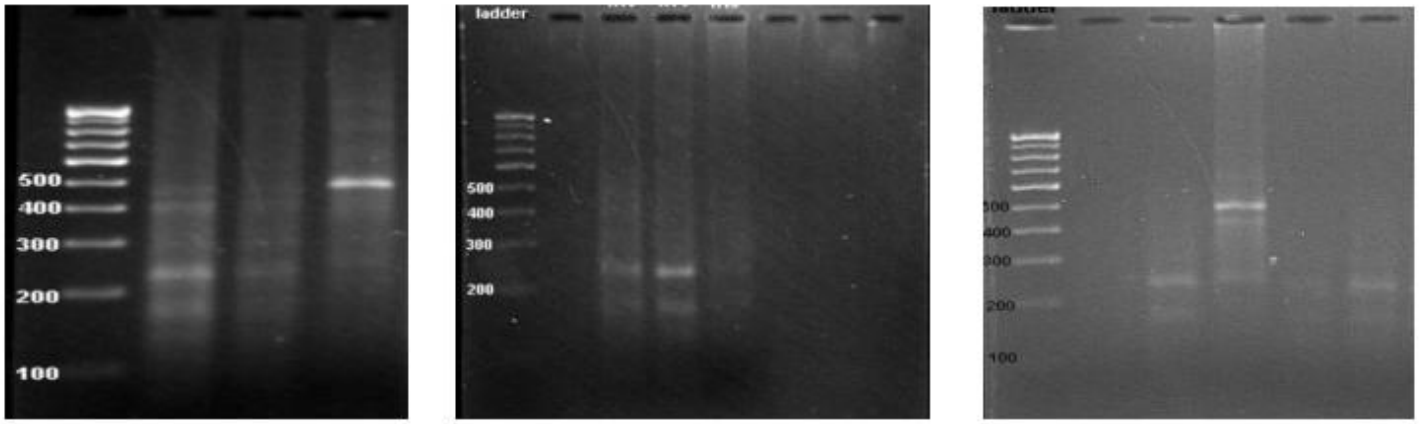

Figure 10: Group C: (Good oral hygiene and absence of dental caries showing incomplete or absence of disease)

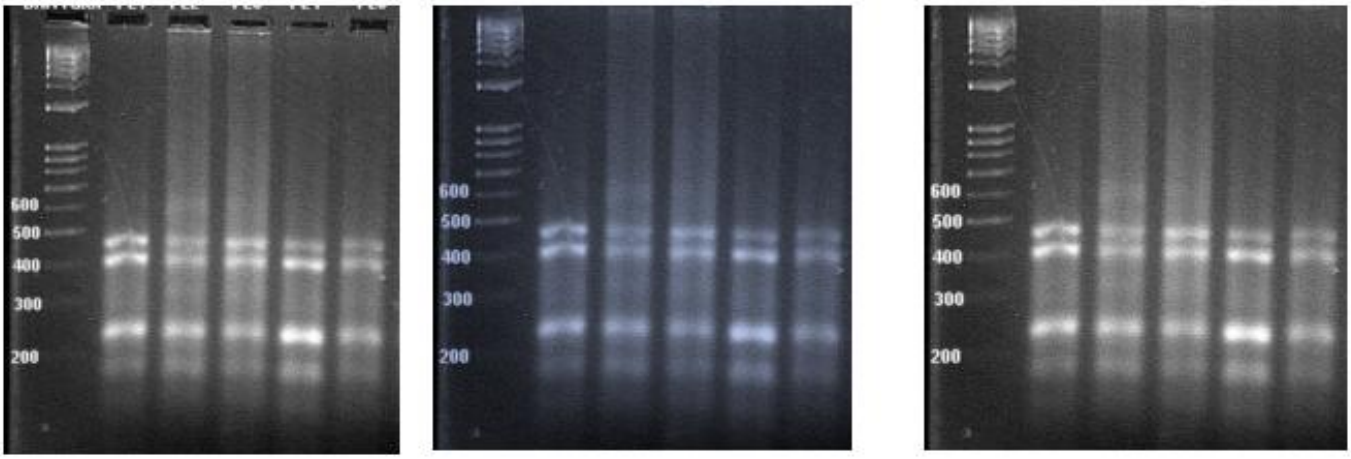

Figure 11: Group D: (Good oral hygiene and presence of caries showing complete digestion at 180 and 250 bp with minor bands)

4. Discussion

Genes play a major role in causation of different types of diseases occurring in our body. The alterations in the structure of genes are responsible for decreased amount of mineral content in enamel.[7] The rationale of this study was to explore an alternative hypothesis for causation of dental caries and emphasize how genetics appears to play a role in the disease process. This study has demonstrated evidence that a single nucleotide polymorphism of MMP-13 gene (rs 2252070) is associated with resistance to the risk for caries progression.

A significant association was seen in group D subjects (patients with good oral hygiene and presence of dental caries). These findings suggest that the polymorphism of MMP-13 gene has a role in causation of dental caries. Two permanent bands were seen at 180 bp and 250 bp along with few minor bands seen after the amplification was digested with specific restriction enzyme Bsr-I. This is in accordance with the study done by Sukalal et al[8] which revealed a wide range of MMP-13 expression level between pulp samples with possible down regulation of MMP-13 expression during caries progression. However, Sukalal's findings are contrary to the study done by Tannure et. Al[9] on a Caucasian population in Brazil and by Loreto et. Al[10]which suggested a role for metalloproteinase-13 in the development and progression of adult human dental tissue disorders.

Group A (poor oral hygiene and absence of dental caries) did not show any association of MMP-13. There was an incomplete or absence of digestion with Bsr-I. It can thus be assumed that there may be different suites of a gene other than MMP- 13, which minimizes the risk of caries developing.

Within Group B (poor oral hygiene and presence of dental caries) the genetic influence was not seen for enabling dental caries. The restriction enzyme showed incomplete or absence of digestion in this group too. The presence of caries may also be due to local factors like plaque or calculus. Due to a paucity of prior research, it is very difficult to comment on the disease process and relate it to genetics contributing to the causation of dental caries in a patient with poor oral hygiene. There may also be an exaggerated response of a certain group of genes which have yet to be explored.

Group C (good oral hygiene and absence of dental caries) had no association for MMP-13 with dental caries. The restriction enzyme showed incomplete or absence of digestion. Maintaining a good oral hygiene is certainly one of the reasons for prevention of dental caries but the genetic component should also be kept under consideration.

The phenotypes in the primary dentition are highly heritable, with genes accounting for 54–70% of variation in caries scores.[11] The heritability of caries scores in the permanent dentition is 35–55% suggesting that dental caries in primary and permanent teeth may be partly attributable to different suites of genes or genes with differential

effects.[11]The phenotypic variation is better explained in permanent than in primary dentition.[12] The subjects having only a permanent dentition were only included in our study. On the contrary Tannureet. et.al[9] studied children and adolescents. This study showed insignificant relations of genetic effects with gender for causation of dental caries (table 2).

Various authors have put forward different methods to analyze the effect of MMPs. In our study, the genomic DNA was obtained from venous blood and genetic polymorphisms in MMP13 gene (rs2252070) were genotyped by polymerase chain reaction PCR using the Taqman method followed by digesting it with specific restriction enzyme Bsr-I. Likewise, different authors have genotyped the gene by using real- time RCR.[9,13,14]

Sulkalal.et. al [8] explained the role of Matrix metalloproteinases (MMPs) and their specific tissue inhibitors (TIMPs) participating in pathological and physiological conditions by using Real-time quantitative polymerase chain reaction followed by western blot and immune histochemical staining.

Considering all the available information and references it can be concluded that MMPs are responsible for tissue repair, break down and remodeling. Although dental caries has multiple etiological variants, the role of a gene cannot be overestimated. Our study reflects the significant effects of a certain gene for the causation of dental caries.

5. Limitation and recommendations

Further studies with larger sample sizes should be carried out for better understanding of genetic predisposition and polymorphism. The small sample size may be bias of the results presented here. Furthermore; gene sequencing has to be done to asses any other related polymorphism other than MMP-13 (rs 2252070).

6. Conclusion

This study strongly suggests that there is an association of MMP-13 (rs 2252050) gene in a group of patients with good oral hygiene and the presence of dental caries. Further genetic based studies should be carried out for better understanding of the causation for dental caries.

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