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ORIGINAL RESEARCH

MUTANS STREPTOCOCCAL TRANSMISSION IN FAMILIES

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ABSTRACT

Introduction: Dental caries is an infectious, transmissible bacterial disease. Although, its modes of transmission include bacterial transfer in between individuals, its transmissibility has been variably reported much less within biological family units. The purpose of this study was to assess Mutans Streptococcal transmissibility from biological parents to child.

Methods: A total of 57 families (comprising of parents and a child) were selected after screening all pediatric subjects visiting the hospital. Age-matched criteria as per WHO index groups were selected: a) Families of children with primary dentition (≤ 6 years) and b) Families of children with permanent dentition (12-16 years). Dental plaque was collected and MS colonies isolated and later on subjected to polymerase chain reaction using gtfB. Data obtained was subjected to descriptive statistics and Chi-Square test.

Results: It was observed that positive correlation (2 bp) was obtained between father-child and mother-child pairs in families of children with carious primary dentitions while no such correlation was obtained among nondetectable caries affecting families of children with permanent dentition. Contrasting results were obtained for noncarious permanent dentitions families which revealed a positive correlation and vice versa.

Conclusion: There was a opposite and different band expression in both dentitions as well as carious states reflecting variations in MS transmissible strains.

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INTRODUCTION

Dental caries is the resultant disease process occurring due to imbalance between enamel demineralization and remineralization. Creation of an acidic niche and its maintenance by acidogenic microflora is a pre-requisite for caries initiation and progression. (Chokshi *et al*., 2016) Formation of biofilm is a complex process involving interactions between bacterial and salivary proteins. This structure allows formation of 3-dimensional structures which help the micro-organisms to develop antibiotic resistance and altering environmental niche. (Krzysciak *et al*., 2014) Mutans streptococcus metabolizes fermentable carbohydrates to produce organic acids which cause a decrease in pH. This reduction leads to enamel solubility which is a feature of dental caries. (Salman *et al*., 2015) Glucosyltransferases (Gtfs) play a significant role in biofilm development. These adsorb onto enamel surface and act as colonization sites for Mutans Streptococcus thus, acting as a virulent source. The Gtfs synthesize insoluble glucans (GTF-B and –C) which act as scaffold for MS aggregation which creates acidic niches that aid in carious destruction. Molecular tools such as- PCR-based assay employs studies on glucosyltransferase genes, 16S RNA, 5' nuclease based RT-PCR, multiplex PCR etc. (Hung *et al*., 2005) The aim of this study was to assess the Mutans Streptococcal similarities in family units comprising of father, mother and a biological child.

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MATERIALS AND METHODS

This was a prospective study designed to screen pediatric subjects and their parents for caries status. Ethical approval for the study was obtained from Institutional Ethical committee. Pediatric cases were age-matched according to WHO index age groups: ≥ 6 years and 12-16 years. A total of 57 biological family units comprising of a father, mother and a child were selected for the study and were categorized into four groups-

a) Families of children with carious primary teeth (n=18)
b) Families of children with no clinically detectable dental caries (n=6)
c) Families of children with carious permanent teeth (n=21)
d) Families of children without non-carious permanent teeth (n=12)

Inclusion criteria for subject selection included-

a) Co-operative and willingness for participation in study.
b) Ability to comprehend instructions for sampling process.

Exclusion criteria included-

a) Antimicrobial therapy.
b) Any systemic disorders that can affect salivary flow.

Assessment of plaque index

Plaque index was assessed using Silness and Loe plaque index criteria (1964) after application of disclosing agent (GC Tripleplaq gel, Fuji, Tokyo).

Collection of plaque sample

Subjects were instructed to refrain from consuming any food or drink two hours prior to sampling. Plaque samples were collected from buccal surfaces of teeth using a cotton-tipped swab. The collected sample was then dispersed in Todd-Hewitt medium and stored at -20°C.

Mutans Streptococcus culture and DNA isolation

Collected samples were plated onto Mitis Salivarius agar and incubated at 37°C in 10% CO_2. Representative colonies were picked up and sub-cultured on Mitis Salivarius agar. MS identification was confirmed by means of Gram's staining. These pure cultures were then stored at -70°C in skimmed milk medium. Aliquots separated from skimmed milk were then plated onto brain heart infusion (BHI) agar and incubated at 37°C in 10% CO_2.

The colonies obtained were then subjected to DNA extraction as per Qiagen protocol (Qiagen assay protocol, Qiagen, Tokyo)

250 µl of each sample was centrifuged. The pellets obtained were resuspended in 570 µl of 20mg/ml 31 Lysozyme solution (Sigma, Tokyo). This was incubated at 37°C for 30 minutes. Post-incubation, 30 µl of 20 mg/ml Proteinase K solution (Qiagen, Tokyo) was added and the obtained solution was incubated at 55°C for 10 minutes duration. To this, 0.8 g of acid-washed glass beads (diameter = 150 X 212 µm, Sigma, Tokyo) and 1 µl of 100 mg/ml RNaseA (Qiagen) were added. The samples were vigorously mixed in 2 ml microtest tubes using a Mixer Mill MM300 (Qiagen) at 30 Hz for 10 minutes. Next,600 µl of buffer AL from a DNeasy tissue kit (Qiagen) was added and incubated at 70°C for 30 minutes. The beads were centrifuged and the supernatant obtained was transferred onto new tubes. Into this supernatant, one-third volume of ethanol was added and mixed.

Primer selection

Primer was designed using BLAST tool on NCBI website. The compositions of forward and reverse primers for gtfB are as follows-

Forward primer: AGCCATGCGCAATCAACAGGTT (GC%=50%)
Reverse primer: CGCAACGCCAACATCTTGATCAG (GC%=52.17%)

Polymerase chain reaction

25 µl of reaction mixture which contained 1X reaction buffer Taq polymerase, 1.5 mM $MgCl_2$, 0.1 mM deoxynucleoside triphosphate, 0.2 µM primer, 1.5 U Taq polymerase along with 2.5 µl DNA sample, was subjected to PCR. The reaction was performed using a Touchgene Gradient (UK) thermocycler. The reaction was performed at optimal temperatures maintained throughout the cycle which was as follows- Denaturation was performed at 95°C for 5 minutes which was followed by amplification cycles (45 cycles) at 95°C for 30 seconds, 36°C for 30 seconds and 70°C for 1 minute. The PCR products obtained were separated by electrophoresis in 1.5% agarose gel. The obtained DNA was stained using 0.5 µg Ethidium bromide. The bands obtained were visualized under UV illumination. Statistical analysis was performed by means of descriptive statistics and employing the Chi-square test for calculation of correlation coefficient.

RESULTS

a) Families of children with primary dentition:

Positive correlations were obtained between father-child and mother-child pairs in families of children with carious teeth (φ=+1) at band size of 2 bp. However, no correlation was obtained between parent-child pairs in families of children with no clinically detectable caries (Table 1a).

b) Families of children with permanent dentition:

No positive correlation was observed among parent-child pairs comprising of children with carious teeth. However, positive correlations between father-child and mother-child pairs were noted in families of children with noncarious dentition (φ= +0.07 and +0.53, respectively) at band size of 1.2 bp (table 1b).

Mutans streptococcus bands for glucosyl transferase B

Table 1(a): Families with children bearing primary dentition

(A) 2 bp

Child with caries

Family member	Band Present	Band absent
Child	1	17
Mother	18	0
Father	18	0

Child without caries

Family member	Band Present	Band absent
Child	0	6
Mother	0	6
Father	0	6

Result

with caries	Φ	without caries	Φ
Father-child	+1	Father-child	-
Mother-child	+1	Mother-child	-

B)1. 2 bp

Child with caries

Family member	Band Present	Band absent
Child	4	13
Mother	0	17
Father	2	15

Child without caries

Family member	Band Present	Band absent
Child	0	6
Mother	1	5
Father	4	2

Results

with caries	Φ	without caries	Φ
Father-child	0	Father-child	-
Mother-child	-	Mother-child	-

(b) Families with children bearing permanent dentition

(A) 1 bp

Child with caries

Family member	Band Present	Band absent
Child	0	21
Mother	1	20
Father	1	20

Child without caries

Family member	Band Present	Band absent
Child	2	10
Mother	4	8
Father	1	11

Results

with caries	Φ	without caries	Φ
Father-child	-	Father-child	+0.07
Mother-child	-	Mother-child	+0.53

DISCUSSION

Clarke (1924) reported involvement of Streptococci in dental caries etiology. (Balakrishnan *et al*., 2000; Bowen and Koo, 2011) Later on, Maclean (1927) confirmed this finding. Abercrombie and Scott (1928) isolated a similar microorganism from blood from a patient suffering from infective endocarditis. (Bowen and Koo, 2011) Keye's and Fitzgerald conducted a series of experiments which revolved around the genetic and microbiological basis of disease. Keyes in his experiments demonstrated that hamsters litters when reared separately, exhibiting variability in caries experience. (Bowen and Koo, 2011) MS colonizes oral cavity only after tooth eruption and forms the major microbial group associated with and responsible for dental caries. (Balakrishnan *et al*., 2000; Lapirattanakul and Nakano, 2014This occurs during a period termed as "Window of Infectivity" which ranges between 19 to 31 months of age (Caufield). It has been theorized that if no colonization takes place during this period, no colonization shall occur before six years of age (concomitant with permanent molar eruption). (Kosai *et al*., 1999) Their colonization levels increase by consumption of sucrose, acid production and attachment to tooth surfaces. Their virulence is strongly associated with carbohydrate consumption. (Balakrishnan *et al*., 2000) MS metabolizes fermentable carbohydrates to produce organic acids which cause a decrease in pH resulting in caries. (Salman and Senthikumar, 2015) MS virulence can be attributed to their adhesion property. The adhesion mechanism involves the primary step (sucrose-dependent) which includes interactions between glucosyltransferases and glucan-binding proteins. (Lapirattanakul and Nakano, 2014) Glucosyltransferases (Gtfs) play a significant role in biofilm development. These adsorb onto enamel surface and act as colonization sites for MS thus, promoting virulence. There are three genetically distinct Gtfs-GtfB, C and D. GtfB binds to bacteria forming tight cell clusters, GtfC adsorbs to enamel while GtfD results in formation of a water-soluble polysaccharide which acts as a primer for GtfB. This polysaccharide matrix affects biofilm virulence by affecting physical and biochemical properties. GtfB synthesizes insoluble glucans that are rich in α-1,3 linkages while GtfD produces predominant soluble glucans. GtfC, on the other hand, synthesizes mixture of soluble (predominantly comprising of α-1,6 linkages) and insoluble glucans. (Bowen and Koo, 2011) These insoluble glycans act as scaffolds for MS aggregation. These are also comprised of glucan-binding domains which aid in glucan binding with various surfaces. The interactions between glucans and cell-associated glucan binding GTF and GBP domains result in MS accumulation within oral biofilms. This accumulation results in production of low pH areas which ensures carious destruction of tooth surfaces. (Smith, 2002) Gtfs can be easily assayed from saliva samples specially, which is obtained from caries-active subjects. (Bowen and Koo, 2011) MS transmission occurs during the first 2.5 years of life. (Tanzer, 1995) An early acquisition of MS forms a central event in natural history of dental caries. This can occur through horizontal or vertical transmission. (Ayilliath *et al*., 2013) Alves *et al* (2009) in their prospective study analyzed S. mutans colonization in 119 children during 1.5 year period. Approximately 50% children with high S. mutans levels caries genotypes which were identical to their mothers. However, non-familial sources of transmission from care-giver-to-child and child-to-child have been reported. However, these investigators in their study did not demonstrate any genotypic matching between caregivers and children. (Tanzer, 1995) Ayilliath *et al*. (2013) correlated S. mutans and Lactobacilli levels between mother and child pairs and found that children of mothers with high S. mutans have an increased risk of dental caries. (Ayilliath *et al*., 2013) Priyadarshini (2013) in their study on 180 mother-child pairs with child age range falling between 3 to 5 years was selected and non-stimulated saliva was obtained for MS culturing. This study also indicated that maternal MS levels are risk indicators for MS levels in children. (Priyadarshini *et al*., 2013)

Oral cavity is inhabited by approximately 52 genotypes of MS. This concurrent and/or coexisting virulence of different MS genotypes determines caries incidence and treatment success and/or failures. (Palmer *et al*., 2012) In current study, positive

correlations in band size of 2bp between father-child and mother-child pairs in carious primary dentition units were observed while no such correlation were observed in family units of children with no clinically detectable caries of primary dentition. Contrasting findings were observed in family units of children with permanent dentition i.e., positive correlation in band size of 1.2 bp between father-child and mother-child pairs with non-carious child dentition and no correlation in family units with carious child dentitions were observed. These findings indicate that different GtfB bands are expressed in primary and permanent dentitions and also, there is difference in band size expressed in both dentition types. These results are unique as no similar findings have been observed previously in any of the published literature available. These observations also provide evidence of MS transmissibility in father-child pairs in family units with carious primary and non-carious permanent child dentitions.

Conclusion

MS can be transmitted among family members through horizontal and vertical transmission. This study has provided evidence that there is indeed such transmission possible within families. There are very few studies which have dealt with paternal-child MS transmissibility molecular level. Hence, this study supports the notion that there is microbial transmission in these lineages as well.

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