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Original Research Article

Mother-Child Pair Transmission of *Streptococcus Mutans*; PCR Based Study in Urban Population of Pakistan

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Abstract: The aim of this study was to determine vertical transmission of *Sterptococcus mutans* (*S. mutans*) genotypes in mother-child pair. The present study was conducted on 60 mother-child pair saliva samples having at least three carious teeth. All enrolled preschool children were divided into two age groups Group-1 (1-3 years) and Group-2 (4-5 years). Stimulated saliva samples were collected from all enrolled subjects. After isolation of *S. mutans* DNA, Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) and genotyping methods (with primers for the glucosyltransferase gene) were used to identify the matching and non-matching patterns of *S. mutans* genotypes transmitted from mother′s to their child. Statistical analysis showed 75% matching genotypes were found in Group-1 mother-child pair and 65 % was reported in Group-2 mother-child pair suggesting the presence of vertically transmitted strains of *S. mutans* higher in Group-1**.**

Keywords: *S. mutans*, Dental caries, Vertical transmission, Arbitrarily Primed Polymerase Chain Reaction (AP-PCR).

INTRODUCTION

Dental caries is the most common infectious disease, and remains to be significant oral health problem worldwide for both children and adults [1]. Dental caries is the result of interaction between microorganisms mainly *Streptococcus mutans* (*S. mutans*), teeth and fermentable carbohydrates [2]. *S. mutans* is a facultative anaerobic Gram-positive cocci that stick to tooth surfaces and break down sugar for energy. This results in lowering of pH and produces acidic environment leading to demineralization of teeth. Continuation of this process initiates the development of dental caries [3].

Dental caries is an infectious process and *S. mutans* is reported to be transmitted both horizontally and vertically [4, 5]. Horizontal transmission is more common in siblings, children in same classroom, nursery or day care centers that have been reported to carry similar bacterial strains in their saliva [6, 7].

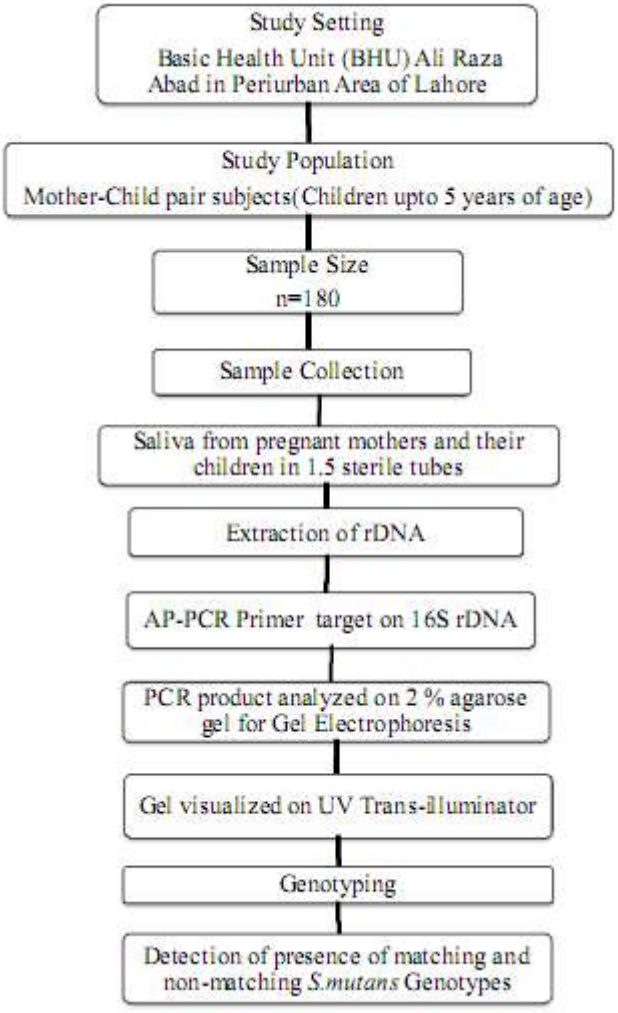
On the other hand, vertical transmission from parents to their children, is restricted by some to genetic transmission and extended by others to include also transmission of infection from one generation to the next, through fluids like saliva, milk and other means [8]. In vertical transmission *S. mutans* spread from mother to their children [9]. Exact method of vertical transmission is still debatable, but it has been reported in the literature that vertical transmission due to close contact between mother and child by sharing of food and utensils [10]. The tendency of transmission is reported to be higher in those children whose mothers are pregnant and have carious teeth with high *S. mutans* count in their saliva [9].

Previous studies using phenotyping or genotyping methods give conflicting evidence. Some studies have suggested that mother is the major primary source of infection for children who carry *S. mutans*

strains (1, 7, 8, 18, 21, 35). However in other studies detection of genotypes that are not found in children's mothers or other family members indicates that *S. mutans* may also be acquired from other sources (7, 8, 18, 28).

Information about the early colonization of *S. mutans* in children's oral cavity could help to explain the development of caries. Thus, this paper presents an observation of the *S. mutans* colonization profiles in healthy Mother-Child group oral cavities, to evaluate the pattern of vertically transmitted *S. mutans* genotypes from mother to child.

MATERIAL AND METHODS



Study design

Study Population

60 mother-child groups' specifically pregnant mothers having two pre-school age group children Group-1 (1-3) and Group-2 (4-5) years of age were selected by convenience sampling. Consent was obtained from mothers for themselves and also for their enrolled children prior to the study. All enrolled children received a dental examination performed by using WHO 1987 caries diagnostic criteria to determine the decayed, missing, filled teeth (dmft) index [11]. Subjects having history of systemic diseases (diabetes, cardiac diseases, hypertension) and administration of antibiotics within 3 months excluded from study. Institutional Review Board (IRB) at Shaikh Zayed Medical Complex approved the study design, protocol, and informed consent.

Sampling

The sample size of the present study was calculated based on the presence of genotypes detection in saliva samples of 60 mother-child group subjects (Group-1 and Group-2) both having at least 3 carious teeth in their oral cavity.

Saliva samples were collected prior to the dental examination of all enrolled children. Subjects stimulated saliva sample was collected in a 1.5 ml sterile eppendorf tube, containing normal saline and stored in cold chain -20^{o} C (isotherm box) within 6 hours and transported to Division of Molecular Virology and Molecular Diagnostics, National Center of Excellence in Molecular Biology (CEMB) University of The Punjab Lahore, for laboratory analysis.

DNA Isolation

Genomic DNA Preparation with NucleoSpin Kit Protocol

DNA extraction was done from stimulated saliva by using the NucleoSpin DNA isolation kit (MHCHERAV NAGEL; Germany) following the protocol given in the kit manual with a few modifications. Briefly.

Polymerase chain reaction Identification

The target gene (16S rDNA) will be amplified with gene specific primers. Nested PCR was used for amplification due to its higher sensitivity and specificity. Isolates were confirmed for species identity in PCR reactions with primers specific for gtfB, encoding glucosyltransferase GTFB-F ($_{5'}$ ACT ACA CTT TCG GGT GGC TTG G$_3$') and GTFB-R ($_{3'}$ CAG TAT AAG CGC CAG TTT CAT C$_{5'}$) , yielding an amplicon of 517 pb for *S. mutans* gtfB gene. Each reaction consisted of 10 µL template DNA, 1µl of each primer, 500 µM of each dNTP, 5 µL 1x PCR buffer, 25 mM MgCl2, and 50/ µL Taq DNA polymerase (Invitrogen, São Paulo, Brazil) in a total volume of 25 µL. The amplification reaction was performed in 30 cycles as follows:

PCR was run at different temperatures to optimize the required genes. PCR amplification was performed in a reaction mixture (20 µl) with 2 units of polymerase enzyme, along with the required reagents, 20 mol of each forward and reverse primers and 20-50 mg of template DNA solution in a thermal cycler (ABI 2700; Applied Biosystem). Thirty thermal cycles were used for the round of PCR. Steps of PCR cycle in fig 1(1) Initial Denaturing at 95 °C for 2 min. (2)

Annealing at 94 °C for 1 min (3) Extension at 72 °C for 1-2 min(4) Final Extension at 72 °C for 10 min.Four cycles are shown here. The blue lines represent the DNA template to which primers (red arrows) anneal that are extended by the DNA polymerase (light green circles), to give shorter DNA products (green lines), which themselves are used as templates as PCR progresses[12].

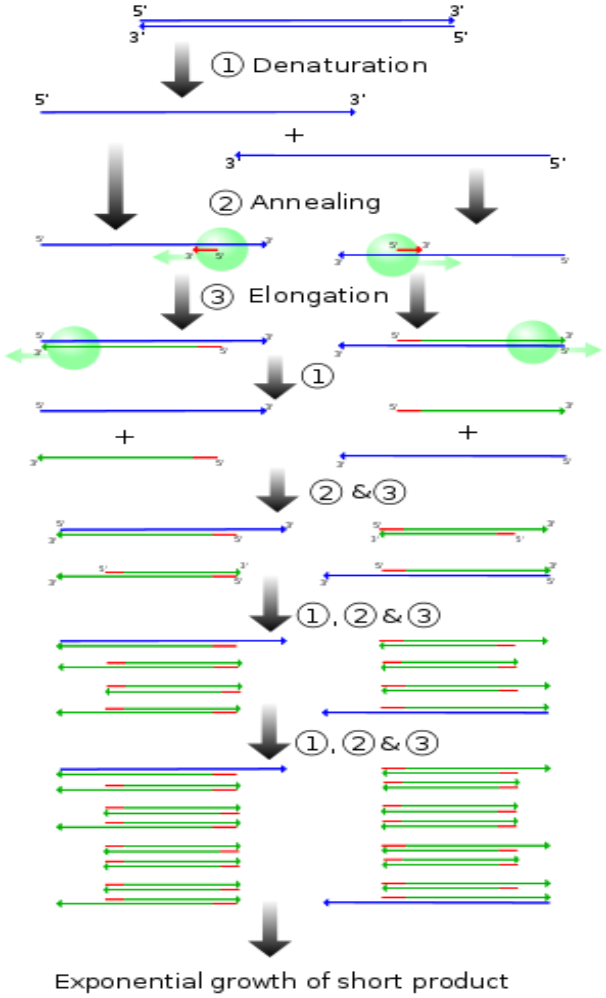


Fig-1: Steps of PCR cycle

Detection of Amplified PCR Product (Agarose Gel Electrophoresis)

PCR products were analyzed on a 2% agarose gel, stained with 30 µlethidium bromide. The bacterial amplification resulted in one band per sample in all cases. A 100 bp DNA ladder served as molecular-size marker in each gel. The desired bands were excised from the gel and purified using the Gel Purification Kit (fermentas). All reactions were repeated twice.

AP-PCR Typing

Strains identified as *S. mutans* were genotyped. The genetic diversity of *S. mutans* isolates was analyzed by AP-PCR reactions. The sequences of the primers OPA 02 (TGC CGA GCT G) and OPA 13 (CAG CAC CCA C) were used. The PCR reactions were performed as follows: PCR buffer with $MgCl_2$ 25 mM, dNTPs 500 µM, 10pmol/µl of each primers (OPA-02 & OPA-13) , Taq DNA polymerase 50/ml, dH_2O and 4.0 µl of DNA sample. The PCR conditions included 35 cycles of denaturation at 94 °C for 1 minutes, annealing at 36 °C for 2 minutes, extension at 72 °C for 2 minutes, with initial denaturation at 94 °C for 5 minutes, and a final extension at 72 °C for 5 minutes. The eletrophoresis was carried out as described previously; however amplification products were analysed in 2% agarose gel.

RESULTS

A total of 180 saliva samples were collected from all enrolled participants. All samples identified *S. mutans* in their oral cavities by doing AP-PCR reaction with specific primers.

The present study reported that 75 % strains of *S. mutans* were transmitted vertically in mother-child group-1, and 65 % strains were transmitted vertically in mother-child group-2 (Table-1). 60 Mother-Child

groups were again divided into 4 subgroups. Each group includes mother and 2 children of both age Groups (1-3 & 4-5). In group A, 34 children of both age groups reported positive vertical transmission of *S. mutans* genes. In group B, 11 children of 1-3 years of age showed presence of vertical transmission and absent in 4-5 years of age children. In group C vertical transmission was present in 4 children of 4-5 years of age but absent in 1-3 years of age. However group D depicts no vertical transmission of *S. mutans* genes in both Groups.

Table 1: Frequency of Vertical Transmission in Mother Child pair Group 1 (1-3 years)& Group-2 (4-5 years)

Sr #	Group-1 (1-3 years)	Group-2 (4-5 years)
	Frequency (%)	Frequency (%)
Valid absent	25	35
Present	75	65
Total	100	100

AP-PCR patterns of *S. mutans* strains isolated from pregnant mothers and their 2-5 years old preschool children and they are detected with OPA-O2 and OPA-13 primers (Figure-2). A 100 bp ladder has been used as a size marker in Lane 1. Mother-child groups with alternate readings. In Lanes 3 and 4 C1 and C2 showed similar pattern with their mother′s pattern M1and M2 in lanes 2 and 5 respectively showed presence of vertical transmission but in M3 (L-8), C1 (L-9) showed similar pattern with mother but C2 (L-11) is different which showed that there might other source of transmission(Figure-3).

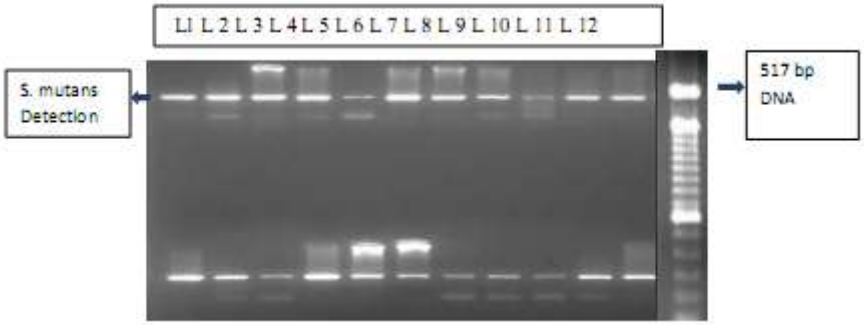


Fig-2: Genotypic detection of *S. mutans* in Mother-Child Pair

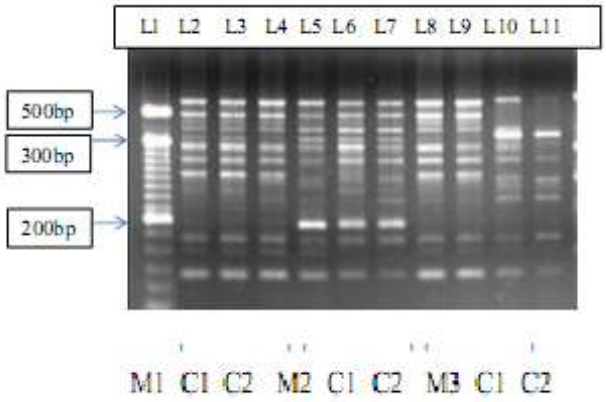


Fig-3: Detection of *S. mutans* Patterns in mother-child pair Saliva Samples

DISCUSSION

Dental caries is a multifactorial transmissible infectious disease that still represents a significant public health problem in many countries [1, 2].

Dental caries is a transmissible disease and *S. mutans* bacterium can be transmitted both horizontally and vertically [5]. The evidence of many studies has shown that most of the children receive the salivary S.mutans mainly from their mothers through saliva exchange by kissing on child mouth or sharing food utensils, spoon. forks and cups. spoon, forks and cups[13, 14]. Straetemans *et al.*, have suggested that delayed acquisition and colonization of *S. mutans* decrease the caries incidence in both primary and permanent dentition of children [15]. Moreover, acquisition of *S. mutans* has been found most commonly during a discrete window period (window of

infectivity) that occurs when the child is at the age of 26 months [16]. Pregnant women are at higher risk of tooth decay that may be due to changes in diet and oral hygiene [17]. One fourth of women of reproductive age have dental caries [18]. Oral health care in pregnancy is often avoided and misunderstood by physicians, dentists, and patients [18]. As evident from research that mothers who had high levels of *S. mutans* in their saliva carry an increased risk of vertically transmitting it to their children [19].Therefore, it is of great importance to detect the presence of *S. mutans* at early years for early diagnosis of dental caries and its treatment.

The present study was conducted on 60 mother-child group saliva samples. Up to 5 years old pre-school children were selected having two age groups , Group-1 (1-3 years) and Group-2 (4-5 years). Reason for selecting pre-school children and splitting them into two groups was that to observe which group has more tendency of vertical transmission of *S. mutans*. There is a frequent contact of saliva from mothers to their children with sharing of food and utensils, and this frequent action causes the early acquisition of *S. mutans* in Pre-school children leading to early childhood caries. In Group-1 75 % vertical transmission was reported and 65 % was reported in Group-2.

With aging the frequency of maternally transmitted strains of *S. mutans* reduced and different strains introduced which showed that in later age *S. mutans* transmitted by other means. So by grouping preschool children it becomes more specified that age Group -1 (1-3 years) is closer to their mother than that of Group-2 (4-5years) age children.

In the present study AP-PCR method was used with 16S rDNA primers to confirm the presence of bacteria in all saliva samples and to observe the transmitted pattern of *S. mutans* genes. This tool provides a more sensitive means of detection of cariogenic bacterial species, as compared with conventional culture techniques [20, 21]. Cultural method is cost effective, gives false positive results due to the presence of cariogenic bacteria *S. sorbinus* in the oral cavity and shows the same characteristic features as the *S. mutans* has [22].

Studies have successfully used dental plaque and saliva as a sample for chromosomal DNA which is extracted by different approaches [23-25]. A Japane study by Oho reported that plaque was not found to be the best dental sample for epidemiological studies as it is difficult to obtain [26]. Keeping these suggestions in mind, the present study implemented the methodology of AP-PCR to detect the presence of *S. mutans* directly from saliva samples similar as a study was conducted in Tanzania by Scheutz and colleagues [27].

CONCLUSION

The result of present study reveals that it is important to observe the early transmission of *S. mutans*genotypes in mother child pair to establish the developmental strategies for the prevention of early childhood caries.

DISCLOSURE STATEMENT

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Enrollment of the study subjects, their data, dmfts score, and sample collection was done at Basic Health Unit Ali Raza Bad in Peri-urban area of Lahore, Pakistan. The molecular analysis of all the study samples was done at Virology laboratory of National Centre of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, Pakistan.We thank all the lady health workers for their support in sample collection and CEMB team for their technical assistance on the part of microbiology.

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