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Association between salivary level of infection with Streptococcus mutans/Lactobacilli and caries-risk factors in mothers

ABSTRACT

Aim *Understanding factors in mothers associated with high and low salivary levels of Streptococcus mutans and Lactobacilli is an important strategy for early childhood caries prevention. Aim of the study was to identify the association between salivary levels of Streptococcus mutans/Lactobacillus and potential caries risk factors in mothers.*

Materials and Methods *Cross-sectional design used a voluntary sample of 300 mothers of young children. Close-ended questions and observations were used to identify mothers' potential caries risk factors. The presence of Streptococcus mutans and Lactobacilli was determined using the CRT bacteria test (Ivoclar Vivadent). All collected information was converted into frequency and proportion describing the prevalence factor in correlation with Streptococcus mutans and Lactobacilli cariogenic bacteria levels of infection.*

Results *Sample participants showed a high caries risk based on socioeconomic, behavioural and clinical factors. Results also showed high levels (>105) of Streptococcus mutans and Lactobacilli infections among 28% of mothers. Three factors were significantly associated with Streptococcus mutans infection: level of education, past caries experiences, and observable dental plaque, whereas, a fourth factor, frequency of daily tooth brushing, was associated to Lactobacilli infection.*

Conclusion *This study showed that easily collectible informations such as maternal level of education, frequency of daily tooth brushing and past clinical factors tend to be associated with high level of Streptococcus mutans and Lactobacilli infections in caregivers.*

Keywords *Caries risk factors; Mothers; Lactobacilli; Streptococcus mutans.*

Introduction

Dental caries is both a public health issue [Ismail, 2004] and a social issue: especially as related to its premature onset in infancy. Dental caries remains a significant oral health problem worldwide for children and adults. It is the dental condition with the greatest clinical and economic impact on an individual over the course of his life [Anti et al., 1994]. Numerous biological, psychosocial and behavioural risk factors are involved in the aetiology of ECC, supporting the multifactorial character of the disease [Berkowitz, 2003]. It is well documented that caries is a transmissible infectious disease in which pathogenic risk factors prevail over protective factors, producing demineralisation of the tooth structure [Gomez et al., 2003]. This disease can occur only if, as a prerequisite, a child is infected by cavity-causing bacteria Streptococcus mutans (S. mutans) in the mouth and its subsequent interaction with sufficient refined sugar and susceptible teeth [Nyvad et al., 2008]. Recent studies have shown that: the infant acquires S. mutans from its mother [Li et al., 1995] although other family members or others in close contact with the child may also be an initial source [Emanuelson, 2001]. The acquisition of S. mutans may occur very early, even before teeth eruption [Miligrom, 2000; Wan et al., 2000; Wan et al., 2003]. The mean age for S. mutans colonisation is 16 months [Wan et al., 2003]. S. mutans and its potential for causing ECC depend in particular on their capacity to adhere, their acido-genicity and their acid-resistence. Caries is a chronic condition affecting mainly children, and preventative action is necessary since it is first established in early childhood [Edestein, 2006]. It has been shown that the premature presence of oral S. mutans in an infant significantly increases his/her risk for dental caries [Miligrom, 2000; Wan et al., 2000; Wan et al., 2003; Köhler et al., 1988; Alalusua and Renkonen, 1983]. Lactobacilli are also implicated as important contributory bacteria in tooth decay and

are late colonisers of the mouth, which may contribute to the demineralisation of the teeth once lesions are established on crown [Tanzer et al., 2001]. The management of this disease continues to be a challenge and requires dental professionals to acknowledge that simply removing or restoring the carious lesion will not change the unhealthy plaque biofilm that contributes to this disease. This demonstrates that the actual cause and risk factors still are not adequately resolved. This can be accomplished through a preventive approach that decreases caries risk factors and increases caries protective factors [Hurlbutt, 2011]. Therefore, given the role of the two bacteria in the onset and progression of caries are inevitably to be considered strategies to protect dental health of children. This can be achieved by intervening initially in improving the dental health of their mothers and caretakers. As an initial step towards improving mothers' dental health, levels of saliva infections with S. mutans and Lactobacilli bacteria have to be determined in order to define the following strategy. Although the presence of ECC in young children is the most single powerful predictive factor of future caries [Pitts et al., 2001], so far there is no validated simple method to detect mothers with high-risk for S. mutans-Lactobacilli. The current maternal bacteria collection and analysis is a complex method that entails delays, costs, requiring laboratory facilities, expertise, material. In a public health perspective, the relevance of developing alternatives to laboratory analysis to identify mothers highly infected with S. mutans and Lactobacilli is more than needed. However, simple methods are now available, such as the CRT bacteria caries risk test (Ivoclar Vivadent), for an accurate and reliable detection of the presence of S. mutans and Lactobacilli. Hence the aim of this study was to investigate the association between mothers S. mutans-Lactobacilli prevalence and caries risk factors based in a model called Maternal Bacteria Risk Transmission Assessment (MBRTA) [Ramos-Gomez, 2010].

Methods

This is as a cross-sectional study, which enrolled 300 mothers from the Main Family Medical Center/ vaccination service in Prishtina/Kosovo, where they went to vaccinate their children. For this study, the ethical approval was obtained from Ethic Committee-Medical Faculty, University of Pristina (Ref. Nr. 1851) and informed consent was obtained from all the participants. Main inclusive criteria defined participants as voluntary mothers who have at least one child aged 12 months or younger (≤ 12 month-olds) and who live with that child for 50% or more of the time. Mothers were first asked to answer a close-ended questionnaire and then saliva samples were collected to determine the level of S. mutans and Lactobacilli infections.

Identification of mothers' potential caries risk factors

Close-ended questions and observations (MBRTA model) were used to identify mothers' potential caries risk factors. Questionnaire items from A to G were used to collect non biological variables, whereas to collect clinical variables the mothers were examined visually at the visit and the results were entered in the items H and I of the questionnaire (Table 1).

Saliva sample

Evidence for measuring S. mutans of mothers of young children was based on previous works [Berkowitz, 2003]. The presence of S. mutans and Lactobacilli was determined using the CRT bacteria caries risk test (Ivoclar Vivadent), which enables the simultaneous determination of the S. mutans and Lactobacilli counts

Variables	Cat.	Lactobacilli ≤105 cfu/ml n (%)	Lactobacilli >105 cfu/ml n (%)	p
A. Yesterday, you had >3 between-meal snacks	Yes	99 (79.2)	26 (20.1)	0.67
	No	142 (81.1)	33 (18.9)	
B. Yesterday, you brushed your teeth less than two times	Yes	54 (72.0)	21 (28.0)	<0.05
	No	187 (83.1)	38 (16.9)	
C. You did not completed your high school	Yes	35 (62.5)	21 (37.5)	<0.05
	No	206 (84.4)	38 (15.6)	
D. You qualify your own caries experiences as very high	Yes	104 (82.5)	22 (17.5)	<0.05
	No	137 (78.7)	38 (15.6)	
E. Within last 12 months, you had dental filling or extraction	Yes	130 (77.8)	37 (22.2)	0.22
	No	111 (83.5)	22 (16.5)	
F. You expect that your child will have a good/excellent oral health	Yes	179 (81.0)	42 (19.0)	0.63
	No	62 (78.5)	17 (21.5)	
G. You consider your own dental health as good or excellent	Yes	134 (84.3)	25 (15.7)	0.06
	No	107 (75.9)	34 (24.1)	
H. At least 1 anterior restoration OR an anterior tooth lost to caries	Yes	90 (72.6)	34 (27.4)	<0.05
	No	151 (85.8)	25 (14.2)	
I. Observable anterior dental plaque	Yes	79 (68.7)	36 (31.3)	<0.05
	No	162 (87.6)	23 (12.4)	
Total		2169 (80.3)	531 (19.7)	

TABLE 1 Correlation between potential caries-risk factors and level of Lactobacilli infection.

in saliva by means of selective agars. The blue mitis-salivarius-agar with bacitracin is used to detect S. mutans, while the light culture medium, Rogosa agar, is used to evaluate Lactobacilli. Stimulated saliva samples were collected from mothers for bacterial assessment. Each subject was given a piece of paraffin pellet, and asked to chew the paraffin and to expectorate the stimulated saliva into the container. Both agars were covered with saliva using a pipette; the carrier was immediately placed in the test vial, which was tightly sealed. After two days of incubation at 37° C / 99° F results were obtained. Findings higher than 10^5 CFU/ml of S. mutans and/or Lactobacilli indicate a high risk for caries disease. The CRT test is sensitive enough to provide a low, medium or high level of cariogenic bacterial challenge. After incubation, the vial used is removed and opened, and the agar carrier is then evaluated using a chart. S. mutans appear as small blue colonies with a diameter of <1 mm on the blue agar, while Lactobacilli appear as white colonies on the transparent green agar. Findings higher than 10^5 CFU/ml S. mutans and/or Lactobacilli indicate a high risk for caries.

Microbiological analyses

Bacterial counts were recorded as Colony Forming Units per milliliter (CFU/ml) of saliva. The number of bacterial colonies was graded as Class: 0, 1, 2, 3 according to the manufacturers' scoring-card. Converting raw data for cross-sectional analytic purposes consisted in scoring S. mutans in terms of its intensity of colonisation, as recommended by Wan et al. [2003]: Group 0: none detected; Group 1: < 10^4CFU/ml; Group 2: 10^4 – 10^5CFU/ml; Group 3: >10^5 CFU/ml

Statistics

The descriptive statistical analyses meet the objectives of this study by first, estimating the prevalence of mothers' cariogenic bacteria using the categorical dichotomised outcomes S. mutans and Lactobacilli. For our purposes, independent variables were presented by categorical frequencies and proportions using statistical tests such as Chi-square. Correlation analysis, in order to achieve our study's goal, exploried the association between the study's outcomes and independent factors converting raw data in yes/no binary categorising. Data analysis was performed using SAS System (SAS Institute Inc.).

Variables	Categories	Total (%)	P
Maternal age	Mean of 28.5 ± 4.3 years	298 (99.0)	<0.0001
Maternal level of education	Primary school completed	47 (15.7)	
	High School completed	112 (37.3)	
	College completed	11 (3.7)	
	University completed	130 (43.3)	<0.0001
Monthly family Income1	< 500 €	157 (52.3)	
	600-1000 €	111 (37.0)	
	1000-2000 €	26 (8.7)	
	2000-3000 €	4 (1.3)	<0.0001
Last time visiting the dentist	< 12 months	175 (58.3)	
	Between 1 and 2 years	89 (29.7)	
	Between 2 and 5 years	17 (5.7)	
	> 5 years ago	16 (5.3)	<0.0001

TABLE 2 Sample of mothers described.

Results

The study included 300 mothers of young children. Mean age of mothers was 28 years, ranging between 23 and 43 years. Almost half of the interviewed mothers reported to have completed their University education (43.3%) and the majority of them stated that their monthly family incomes are in lower categories (Table 2).

The vast majority of participants tend to be S. mutans and Lactobacilli infected as shown in Table 3. Participants tend to be more highly infected by S. mutans than Lactobacilli. About 30% of the mothers were highly S. mutans infected; if the threshold is set at 10^4 CFU/ml, this proportion doubles (60%). The categories of infection with values of 10^2 - 10^5 CFU/ml tend to be similar in terms of S. mutans or Lactobacilli infected ratio.

The homogeneity of mothers' answers distribution with respect of the level of S. mutans and Lactobacilli

Categories	S. mutans N (%)	p	Lactobacilli N (%)	p
General distribution				
Group 0: non detected	29 (9.7 %)		36 (12.0 %)	
Group 1: 10^2–10^3 cfu/ml	90 (30.0 %)		96 (32.0 %)	
Group 2:10^4–10^5 cfu/ml	97 (32.3 %)	<0.05	109 (36.3%)	<0.05
Group 3: > 10^5 cfu/ml	84 (28.0 %)		59 (19.7 %)	
Specific distribution				
Values ≤ 10^5 cfu/ml	216 (72.0 %)		241 (80.3%)	
Values > 10^5 cfu/ml	84 (28.0 %)	<0.05	59 (19.7 %)	<0.05

TABLE 3 General and specific distribution of S. mutans and Lactobacilli levels among mothers.

binary threshold of infection is shown in Table 1 and 4. The trend in p-value for S. mutans and Lactobacilli correlated to caries-risk factors generated almost similar results (level of significance at 0.05) except for two risk factors. High level of infection with S. mutans is associated with three statistically significant caries-risk factors (level of education, anterior caries experiences and observable dental plaque).

Answers about behaviours associated to potential caries-risk factors represent 51% of all answers: snacking behaviour, self-perceived caries experiences, recent dental treatment, self-perceived oral health and observable dental plaque were the most frequent (>40%) potential caries-risk factors (Tables 1 and 4).

Discussion

The study results show a high percentage of mothers infected with S. mutans and Lactobacilli, with a higher percentage of the former than the latter. Salivary S. mutans were detected in 90.3% and Lactobacilli in 88.0% of the mothers. The percentage of the mothers free of salivary S. mutans was 9.7% and for Lactobacilli was 12.0%. Mothers infected with S. mutans with values >10^5 CFU/ml was 28% and those infected with Lactobacilli were 19.7% (Table 3). In the study, the main potential caries risk factors associated with high level of infection with S. mutans and Lactobacilli appeared to be items B, C, G, H, I.

Variables	Cat.	S. mutans ≤105 cfu/ml n (%)	S. mutans >105 cfu/ml n (%)	p
A. Yesterday, you had >3 between-meal snacks	Yes	91 (72.8)	34 (27.2)	0.79
	No	125 (71.0)	50 (28.6)	
B. Yesterday, you brushed your teeth less than two times	Yes	51(68.0)	24 (32.0)	0.37
	No	165 (73.3)	60 (27.0)	
C. You did not complete your high school	Yes	27 (48.2)	29 (51.8)	<0.05
	No	189 (77.5)	55 (22.5)	
D. You qualify your own caries experiences as very high	Yes	92 (73.0)	34 (27.0)	0.74
	No	124 (71.3)	50 (28.7)	
E. Within last 12 months, you had dental filling or extraction	Yes	114 (68.3)	53 (31.7)	0.10
	No	102 (77.7)	31(23.3)	
F. You expect that your child will have a good/excellent oral health	Yes	161 (72.8)	60 (27.1)	0.58
	No	55 (69.6)	24 (30.4)	
G. You consider your own dental health as good or excellent	Yes	116 (72.9)	43 (27.0)	0.69
	No	100 (71.0)	41 (29.0)	
H. At least 1 anterior restoration OR an anterior tooth lost for caries	Yes	82 (66.1)	42 (33.8)	<0.05
	No	134 (76.1)	42 (23.8)	
I. Observable anterior dental plaque	Yes	72 (62.6)	43 (37.4)	<0.05
	No	144 (77.8)	41 (22.2)	
Total		1944 (72.0)	756 (28.0)	

TABLE 4 Association between potential caries-risk factors and level of S. mutans infection.

Item B - Tooth brushing less than 2 times a day: This was considered an inadequate exposure to fluoride [Duggal and Loveren, 2001]. In the study of Domejean-Orliaguet [006] fluoride toothpaste use in adults (odds ratio, OR =0 .7) was negatively related to cavitation risk.

Item C - Level of education lower than high-school: In adults, the level of education is a robust indicator of the Socio-Economic-Status (SES) [Armfield and Spencer, 2004]. Paulander and Lindhe [2003] showed that a low level of education is associated with poor oral health conditions and this should be considered in assessing caries risk (and in planning appropriate preventive measures). Colonisation of S. mutans in early childhood has been related to the lower SES of families.

Item G - Caregiver opinion on her mouth: This question was based on studies on locus of control associated to caries risk.

Item H - Past caries on anterior teeth or on smooth surfaces: Previous studies [Broadbent and Poulton, 2006] consistently identified a correlation between past and future caries.

Item I - Visible dental plaque: Berkey [2006] showed that plaque accumulation is positively associated with a more rapid caries progression.

Presence of salivary Lactobacilli is comprised among four significant caries risk factors, which include frequency of daily tooth brushing, level of education, anterior caries experiences and observable dental plaque, as well as another one almost statistically significant: oral health self-perception. A study conducted on different age groups (children) showed similar results and it confirmed that presence of Lactobacilli could be considered a strong indicator for caries [Martínez-Pabón et al., 2010]. Unlike infection with Lactobacilli, infection with S. mutans is associated with less significant caries risk factors (such as: level of education, anterior caries experiences and observable dental plaque). Infact, the study conducted by Martínez-Pabón [2010] shows that the presence of S. mutans is not correlated with caries in children.

Mothers' level of education and clinical observations seem to be the factors most frequently associated to high level of infection with both S. mutans and Lactobacilli.

The low level of education of mothers was also one of the potential risk factors in other studies. In two separate studies by Begzati and Priyadarshinil it was found that

the dmft index of the children whose mothers had a higher level of education was significantly lower than that of the children whose mothers had a lower level of education [Begzati et al., 2010; Priyadarshinil et al., 2013].

Our study results show that questions related to behaviours (snacking and tooth brushing) do not seem to be associated to the S. mutans level of salivary infection.

These initial results can be utilised by other researchers developing scientific evidences on the same topic.

The analysis of risk factors, such as maternal level of education and anterior caries experience, can be used as a simple method to detect a high level of infection in mothers, thus avoiding the costs of laboratory analysis and tests for public health institutions.

Moreover, the knowledge of these trends would be useful in developing preventive strategies for caries.

The data presented in this study, have internal validity in Kosovo in terms of mother's profile and can be similar in other South Eastern Europe countries.

Conclusion

This study showed that easy collectible data such as maternal level of education, frequency of daily tooth brushing and anterior caries experience tend to be associated to high levels of S. mutans and Lactobacilli infections. These factors could be considered to determine the level of infections with S. mutans and Lactobacilli bacteria without the need to use commercial tests.

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