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Cariogenic bacteria and dental health status in adolescents: the role of oral health behaviours

ABSTRACT

Aim *To evaluate the association between dental health status and levels of cariogenic bacteria in teenagers and the influence of behaviours and socio-demographic background on levels of bacteria.*

Materials and methods *Study Design: A cross-sectional population-based sample of 13-year-old adolescents (112 females and 78 males, total 190) was examined. The number of decayed, missing and filled surfaces (DMFS), plaque and hygiene index were recorded according to the WHO criteria. The saliva samples were collected in a sterile container and then analysed by culture on Mitis Salivarius Bacitracin (MSB) agar for mutans Streptococci and on Man Rogosa Sharp (MRS) agar for Lactobacilli. The levels of bacteria were expressed as the number of colonies forming units per millilitre of saliva (CFU/ml). Associations between levels of mutans Streptococci and Lactobacilli and dental health were estimated by odds ratio (OR) and 95% confidence interval (95%CI) using unconditional logistic regression.*

Results *No mutans Streptococci were detected in 53.2% of the adolescents but 22.6% presented ≥103 CFU/ml. For Lactobacilli, these values were, respectively 43.7% and 34.7%. After adjustment for gender and social class, the OR (95% CI) for DMFS >5 was 8.66 (3.57-21.02) if mutans Streptococci ≥103 CFU and 2.11 (0.96-4.64) if Lactobacilli ≥10^3 CFU.*

Conclusion *This data allow us to conclude that hygiene habits and dental visits are not associated with high levels of cariogenic bacteria, but high scores of DMFS were found in adolescents with high levels of mutans Streptococci and lower parents' education.*

Keywords *Caries; Lactobacilli; mutans Streptococci; Oral hygiene; Population-based.*

Introduction

The second half of the twentieth century saw changes in oral health which are unmatched in history. Preventive programmes promoted by the World Health Organization (WHO) in 1981 included oral hygienists and dentists working for community health to offer preventive care such as fissure sealing, oral hygiene instruction, fluoride supplements, all over the world, including Portugal [Almeida et al., 2003]. Despite this, dental caries is still a public health problem with high prevalence and great socio-economic impact that have led to some changing in the goals, objectives and targets advocated by the WHO for the future years [Petersen, 2009; Hobdell et al., 2003]. The promoted strategies reinforce the need to tailor interventions to the local settings such as adequacy of the basic information, local priorities and oral health systems, as well as prevalence and severity of diseases and socio-environmental conditions [Petersen, 2009; Hobdell et al., 2003]. Most epidemiological studies on risk factors for caries in children and adolescents have focused their attention on social, demographic and behavioural factors [Petersen, 2009; Petersen, 2005]. They found worse oral health conditions in those with lower socio-economic status, mainly attributed to the poor hygiene habits and limited access to health care and information [Petersen, 2009; Antunes et al., 2002].

Salivary levels of *Streptococcus mutans* and *Lactobacillus* spp. have also been regarded as primary causative agents of dental caries in humans [Buischi et al., 1989; Zickert et al., 1982; Klock et al., 1977; Beighton et al., 1996]. However the role of behavioural and demographic characteristics in the pathway between microbiological agents and caries is not completely understood, in particular in adolescents.

The aim of this study was to evaluate the association between dental health status and levels of cariogenic bacteria in teenagers and the influence of oral health behaviours and socio-demographic background in levels of bacteria.

Material and Methods

Study population

The Department of Clinical Epidemiology, Predictive Medicine and Public Health of the University of Porto began a cohort named Epiteen (Epidemiological Heath Investigation of Teenagers in Porto), comprising adolescents born in 1990 and enrolled in public (state administered) and private school (not administered by local or national governments) of Porto [Mello et al., 2008]. The Ethics Committee of the University Hospital of São João, Porto, approved the study. Written informed consent was obtained both from parents and adolescents prior to the study.

Collection of data comprised two structured questionnaires; one was answered by parents and adolescents, at home; the other was completed by the adolescents at school. The home questionnaire assessed family characteristics, namely parents' education and adolescents' visits to the dentist. The questionnaire filled at school comprised questions related to the adolescent's behaviours, such as oral hygiene habits. The educational attainment of parents was measured by the number of years of formal schooling, and the adolescent was classified according to the higher score of parents.

Dental examinations were performed at schools, for 700 adolescents, corresponding to the former one third of the whole cohort to be enrolled in the study as previously described [Mello et al., 2008]. Because of laboratory limitations in the maximum number of samples analysed, among the adolescents with dental examination we randomly selected a sample in each school. So, we obtained saliva samples for determination of *mutans Streptococci* and *Lactobacilli* levels, for 207 adolescents. Comparing the 207 adolescents with saliva sample with those with dental examination, we found statistical differences only for the proportion of adolescents enrolled at private schools and the proportion of females, both higher in participants with saliva samples (data not showed). From those with saliva sample we excluded 17 subjects because they reported antibiotic intake in the 30 days prior to collection. So, the final sample comprised 190 adolescents, 78 males and 112 females.

Clinical examination

The number of decayed, missing and filled surfaces (DMFS), plaque index and hygiene index, the OHI-S [Greene and Vermillion, 1964] was recorded for each subject after oral examination using light and exploration using dental mirrors according to the WHO criteria [World Health Organization, 1997]. The same dentist, assisted by one recording clerk, performed all examinations. The dentist was trained and calibrated for application of the WHO guidelines. The intra-observer kappa statistics was 0.876, which is considered good agreement.

Collection of samples for microbial analysis

Participants contributed with 10 ml of saliva stimulated by chewing a piece of paraffin wax (approximately 1.5 g) for 5 minutes. Samples were collected in sterile vials and transported to the laboratory in ice container within less than 30 minutes.

Laboratory procedures

Samples were vortexed for 30 seconds at the maximum setting to uniformly mix the saliva in a cyclomixer vortex (DG-Mx-27, Grifols S.A.) and ten-fold serially diluted in 0.9 ml of TGY broth. Using a sterile bent rod, 0.1 ml aliquots from dilutions 10-1 and 10-2 were plated on Mitis Salivarius Bacitracin agar (MSB, Difco, Laboratories Detroit, MI) selective for *mutans Streptococci* and the same volume on Man Rogosa Sharp (MRS- Oxoid) selective for *Lactobacilli*.

The MSB and MRS plates were incubated at 37 °C in 10% CO_2 for 48–72 hours.

Colonies on MSB agar exhibited morphological characteristics similar to the reference strain (*Streptococcus mutans* CIP 103220) under light stereomicroscope (amplification 50x) were counted and presumptively identified as described previously [McGhee et al. 1982]. *Lactobacilli* were identified as Gram-positive, catalase negative rods [Beighton et al., 1989].

Colonies enumeration was expressed by the number of colonies forming units per millilitre (CFU/ml) of saliva.

The adolescents were classified into three categories according to the levels of *mutans Streptococci* and *Lactobacilli*: 0, 10^1-10^3 and $>10^3$ per millilitre of saliva.

Data analysis

For data analysis we used the software Statistical Package for the Social Science (SPSS – PC Version 14, Chicago, IL, USA). A significance level of 5% was considered.

The chi-square test was used to determine the association between levels of *mutans Streptococci* and *Lactobacilli*, dental health status, socio-demographic characteristics and oral health behaviours. Associations between levels of *mutans Streptococci* and *Lactobacilli* and dental health were estimated by odds ratio (OR) and 95% confidence interval (95%CI) using unconditional logistic regression. We have considered dental health, for individuals who expressed a DMFS index ≤5, considering that equivalent to all affected surfaces of one posterior tooth, and plaque index <1 [Schuller et al., 2001].

Results

In our sample the median (percentile 25-75) DMFS was 3.00 (0.00-8.75) in girls and 1.00 (0.00-5.25) in boys (p=0.015). For plaque index the values were 1.00

Levels of Mutans Streptococci	0	10^1-10^3	>10^3	p-value*
	n=101	n=46	n=43	
Socio-demographic characteristics	n (%)	n (%)	n (%)	
Gender				
Girls	52 (46.4)	34 (30.4)	26 (23.2)	
Boys	49 (62.8)	12 (15.4)	17 (21.8)	0.036
Parents education (years)				
0 - 6	12 (25.0)	16 (33.3)	20 (41.7)	
7 - 9	19 (51.4)	7 (18.9)	11 (29.7)	
More than 10	62 (69.7)	19 (21.3)	8 (9.0)	<0.001
Missing	8	4	4	
School type				
Public	43(42.6)	31(67.4)	38(88.4)	
Private	58(57.4)	15(32.6)	5(11.6)	
BEHAVIORAL FACTORS				
Dental visits				
Never or only when has a problem	30 (47.6)	21 (33.5)	12 (19.0)	
1 a 2 X per year	35 (57.4)	10 (16.4)	16 (26.2)	
More than 2 X per year	35 (53.8)	15 (23.1)	15 (23.1)	0.297
Missing	1	0	0	
Brushes the teeth				
More than2 X per day	32 (50.0)	18 (28.1)	14 (21.9)	
2 X per day	45 (52.9)	19 (22.4)	21 (24.7)	
1 X per day or less	23 (59.0)	9 (23.1)	7 (17.9)	0.834
Missing	1	0	1	
Dental floss use				
Never	68 (49.6)	37 (27.0)	32 (23.4)	
1 X per week	12 (52.2)	61 (26.1)	5 (21.7)	
More than 1X per week	20 (71.4)	3 (10.7)	5 (17.9)	0.292
Missing	1	0	1	
Change brush				
At least 1X in 3 months	36 (51.4)	16 (22.9)	18 (25.7)	
1 X in 3 months	35 (50.7)	21 (30.4)	13 (18.8)	
More than 3 in 3 months	14 (58.3)	8 (33.3)	2 (8.3)	
Don´t know or Missing	16 (59.3)	1 (3.7)	10 (37.0)	0.052
DENTAL HEALTH STATUS				
Plaque índex				
0 - 0.99	35 (56.5)	14 (22.6)	13 (21.0)	
1.00 - 1.99	52 (55.9)	24 (25.8)	17 (18.3)	
≥ 2.00 - 3.00	14 (40.0)	8 (22.9)	13 (37.1)	0.223
Hygiene índex				
0 - 0.99	35 (58.4)	14 (23.3)	11 (18.3)	
1.00 - 1.99	42 (55.3)	19 (25.0)	15 (19.7)	
De 2.00 - 2.99	18 (46.2)	10 (25.6)	11 (28.2)	
≥ 3.00	6 (40.0)	3 (20.0)	6 (40.0)	0.523
DMFs				
0	45 (73.8)	13 (21.3)	3 (4.9)	
1 - 5	40 (56.3)	21 (29.6)	10 (14.1)	
6 -10	11 (35.5)	7 (22.6)	13 (41.9)	
> 10	5 (18.5)	5 (18.5)	17 (63.0)	<0.001
* p-value for the comparison between the three categories (Chi-square test or Fisher exact test)				

TABLE 1 Comparison of the levels of mutans streptococci according to dental health status, socio-demographic characteristics and behavioural factors.

(0.67-1.66) and 1.00 (0.67-1.54), respectively for girls and boys (p=0.748). Hygiene index was 1.25 (0.71-2.17) in girls and 1.00 (0.67-1.88) in boys (p=0.401). Brushing teeth at least twice a day was significantly more frequent in girls (90%) than in boys (64.1%, p<0.001).

Mutans Streptococci were not detected in 101 (53.2%) of the adolescents, 46 (24.2%) presented 10^1 to 10^3 CFU and 43 (22.6%) had >10^3 CFU per ml saliva. Six (3.2%) adolescents presented levels higher than 10^6 CFU/ml. We found higher levels of *mutans Streptococci* in girls, in adolescents enrolled at public schools, according with decreasing parent's education and with increasing in DMFS (Table 1). No differences were found regarding the behavioural factors (Table 1).

No *Lactobacilli* were detected in 83 (43.7%) of the adolescents. Forty-one adolescents (21.6%) presented 10^1 to 10^3 CFU and 66 (34.7%) presented ≥10^3 CFU, whereas 41 (21.6%) had values ≥10^5 CFU/ml. Despite the higher levels of *Lactobacilli* found on adolescents in public schools and the accordingly increased values of DMFS, no other differences were found (Table 2).

After adjustment for gender and type of school, we found a positive and strong association between higher levels of *mutans Streptococci* and DMFS higher than 5 (OR=8.66; CI 95%, 3.57-21.02); however no significant association was found with plaque index. A dose-effect relationship was found between *Lactobacilli* and dental caries or dental plaque, but neither of them reached a statistically significant association (Table 3).

Discussion

In general, our results suggested that higher levels of cariogenic bacteria are associated with worse dental health, which are related with socio-demographic characteristics but not with oral hygiene habits or visits to the dentist.

We have found a stronger association between levels of *mutans Streptococci* and DMFS than *Lactobacilli* even after adjustment for sex and type of school. These results are in agreement with other studies and support the role of *mutans Streptococci* in caries aetiology [Zickert et al., 1982; Kohler et al., 1995; Aguilera-Galaviz et al., 2005; Llena-Puy et al., 2000].

Although access to dental care and tooth brushing is strongly recommended by WHO and oral health professionals as one of the main methods of elimination of dental plaque and for prevention of gingivitis and dental caries, the role of oral hygiene in caries prevention is unclear [Petersen, 2009; Sutcliffe, 1996]. We did not find a significant association between levels of bacteria and visits to the dentist or oral hygiene habits neither when we considered the clinical oral hygiene index or the questions about oral hygiene habits. These results are in agreement with our previous results that did not find a statistical association between these behaviours and caries [Mello et al., 2008].

We found higher DMFS scores in females than males, even if females reported more regular oral hygiene practices and presented similar plaque index than boys. This result lso reinforce the fact that regular hygiene habits do not solve the problem of cariogenic bacteria. The differences regarding salivary levels of *Lactobacilli* according to gender had not been consistent in all studies [Zickert et al., 1982]. However one probable explanation could be hormonal fluctuations, particularly in puberty, that favour the development of cariogenic bacteria in females [Zusman et al., 2005; Lukacs et al., 2006]. Since our participants were 13 years old, age at which in girls puberty is often complete, while in boys puberty usually ends later, most of the difference found between genders in our sample might be related with those hormonal differences.

In our study we have found a significant inverse relationship between parents' education and levels of mutans Streptococci. The same relationship was found with the type of school, another social indicator. Similar results were found regarding the levels of *Lactobacilli*, although without statistical significance. Those results could contribute to explain the higher prevalence of dental caries in those with lower socio-economic status [Petersen, 2005; Antunes et al., 2002; Mello et al., 2008]. Nevertheless the worse oral health in those with lower socio-economic conditions is usually attributed to worse hygiene habits and health care access; our results did not support this approach since in our data the visits to the dentist and hygiene habits were not associated with bacteria levels or dental caries. However, the family context of the child or the adolescent had a great importance as the family represents the children's primary source of background on oral health. So, if they are informed about the aetiology of the disease, they could act to prevent the colonisation by cariogenic bacteria with other behaviours beyond those evaluated in our study [Petersen, 2005; Antunes et al., 2002; Zusman et al., 2005; Lukacs et al., 2006; Peres et al., 2009].

A limitation of our study was the inability to collect saliva samples for all adolescents that performed the oral examination. This limitation results from laboratory restrictions of the maximum number of samples in each day. Besides in each school we randomly selected the students undergoing saliva collection, and as we always collected the maximum number of samples, it resulted a larger proportion of adolescents from private schools. This could weaken the associations found, which is particularly relevant to *Lactobacilli* because we are unable to understand if the not significant association with caries could be explained by this selection bias.

In the last years a significant reduction in caries prevalence took place in Portugal [Almeida et al., 2003], nevertheless we found a high prevalence of

TABLE 2 Comparison levels of lactobacilli according dental health status, socio-demographic characteristics and behavioural factors.

Levels of Lactobacilli	0	10^1-10^3	>10^3	p-value*
	n=83	n=41	n=66	
Socio-demographic Characteristics	n(%)	n(%)	n(%)	
Gender				
Girls	46 (41.1)	26 (23.2)	40 (35.7)	
Boys	37 (47.4)	15 (19.2)	26 (33.3)	0.657
Parents education (years)				
0 - 6	17 (35.4)	11 (22.9)	20 (41.7)	
7 to 9	15 (40.5)	10 (27.0)	12 (32.4)	
More than 10	44 (49.4)	15 (16.9)	30 (33.7)	0.453
Missing	7	5	4	
School type				
Public	32(38.6)	27(65.9)	53(80.3)	
Private	51(61.4)	14(34.1)	13(19.7)	<0.001
BEHAVIOURAL FACTORS				
Dental visits				
Never or only when has a problem	25 (30.1)	13 (32.5)	25 (37.9)	
1 a 2 X per year	34 (55.7)	13 (21.3)	14 (23.0)	
More than 2 X per year	24 (36.9)	14 (21.5)	27 (41.5)	0.156
Missing	0	1	0	
Brushes the teeth				
More than 2 X per day	27 (42.2)	17 (26.6)	20 (31.3)	
2 X per day	35 (41.2)	15 (17.6)	35 (41.2)	
1X per day, occasionally	21 (53.8)	8 (20.5)	10 (25.6)	0.330
Missing	0	1	1	
Dental floss use				
Never	63 (46.0)	28 (20.4)	46 (33.6)	
1 X per week	6 (26.1))	6 (26.1)	11 (47.8)	
More than 1X per week	14 (50.0)	6 (21.4)	8 (28.6)	0.431
Missing	0	1	1	
Change brush				
At least 1X in 3 months	30 (42.9)	18 (25.7)	22 (31.4)	
1 X in 3 months	30 (43.5)	13 (18.8)	26 (37.7)	
More than 3 in 3 months	11 (45.8)	7 (29.2)	6 (25.0)	
Don´t know or Missing	12 (44.4)	3 (11.1)	12 (44.4)	0.593
DENTAL HEALTH STATUS				
Plaque index				
0 - 0.99	34 (54.8)	12 (19.4)	16 (25.8)	
1.00 - 1.99	33 (35.5)	24 (25.8)	36 (38.7)	
≥ 2.00 - 3.00	16 (45.7)	5 (14.3)	14 (40.0)	0.129
Hygiene index				
0 - 0.99	33 (55.0)	11 (18.3)	16 (26.7)	
1.00 - 1.99	28 (36.8)	18 (23.7)	30 (39.5)	
2.00 - 2.99	18 (46.2)	9 (23.1)	12 (30.8)	
≥ 3.00	4 (26.7)	3 (20.0)	8 (53.3)	0.284
DMFs				
0	33 (55.0)	13 (21.3)	18 (29.5)	
1 - 5	28 (36.8)	15 (21.1)	19 (26.8)	
6 -10	18 (46.2)	9 (29.0)	14 (45.2)	
> 10	4 (26.7)	4 (14.8)	15 (55.6)	0.051
* p-value for the comparison between the three categories (Chi-square test or Fisher exact test)				

Participants characteristics	Crude	Adjusted*
	OR (95% CI)	OR (95% CI)
	DMFS (≤ 5 vs. > 5)	
Gender		
Female	1	1
Male	0.60 (0.32-1.15)	0.65 (0.33-1.28)
School type		
Public	1	1
Private	0.23 (0.11-0.48)	0.23 (0.11-0.49)
Mutans Streptococci (CFU/ml)		
0	1	1
10^1-10^3	1.87 (0.80-4.38)	1.37 (0.56-3.35)
>10^3	12.26 (5.28-28.45)	8.66 (3.57-21.02)
Lactobacilli (CFU/ml)		
0	1	1
10^1-10^3	1.94 (0.83-4.57)	1.40 (0.57-3.44)
>10^3	3.28 (1.58-6.81)	2.11 (0.96-4.64)
	Plaque index (< 1.0 vs. ≥ 1.0)	
Gender		
Female	1	1
Male	0.86 (0.46-1.59)	0.89 (0.48-1.66)
School type		
Public	1	1
Private	0.64 (0.35-1.18)	0.65 (0.35-1.20)
Mutans Streptococci (CFU/ml)†		
0	1	1
10^1-10^3	1.21 (0.57-2.57)	1.06 (0.49-2.33)
>10^3	1.22 (0.57-2.64)	0.99 (0.43-2.28)
Lactobacilli (CFU/ml)†		
0	1	1
10^1-10^3	1.68 (0.75-3.74)	1.57 (0.69-3.58)
>10^3	2.17 (1.06-4.42)	1.98 (0.92-4.25)
*Adjusted for type of school and gender; †CFU/ml: number of colonies forming units per millilitre.		

TABLE 3 Risk estimated of caries (DMFS > 5) and plaque index (≥ 1.0) according levels of mutans streptococci and lactobacilli.

caries [Mello et al., 2008]. Our current data show an important role of cariogenic bacteria, namely mutans Streptococci, specifically in adolescents from lower socio-economic class and not explained by oral hygiene habits. This results support the need to improve strategies on oral health promotion particularly in individuals from less advantaged backgrounds and focusing the role of cariogenic bacteria.

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Conflict of interest

The authors declare that they have no conflict of interest.

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