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Differences of the oral colonization by Streptococcus of the mutans group in children and adolescents with Down syndrome, mental retardation and normal controls

Alfredo G Linossier [1], Carlos Y Valenzuela [2], Héctor Toledo [3]

(1) Servicio de Odontología y Cirugía Maxilo Facial, Escuela de Medicina, Pontificia Universidad Católica de Chile
(2) Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile
(3) Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile

Correspondence:
Dr. Alfredo G Linossier
Gran Avenida 6477, Depto 208,
Santiago (La Cisterna), Chile
E-mail: alinossi@gmail.com

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Abstract

Objective: to compare the concentration and serotype of *Streptococcus mutans* in saliva of Down syndrome (DS), mental retarded (MR) and healthy control (C) individuals of the Región Metropolitana Sur of Santiago of Chile. Design of the study: Hundred and seventy nine male and females children and adolescents, aged between 5 to 19 years, 59 DS, 60 MR and 60 C were studied. Saliva samples were cultured in TYCSB agar for quantification, biochemical and serological tests. ANOVA and Chi-square for homogeneity tests were applied. Results: C, DS and MR presented *Streptococcus mutans* (serotypes c, e, f) and *Streptococcus sobrinus* (d, g, h), but only among DS and MR non-typifiable (No-tip) *Streptococcus mutans* were found. MR and DS showed higher bacteria concentration scores than C (P=0.001). Serotypes showed a significant heterogeneity of concentration scores: d, g, h showed the highest and No-tip the lowest one (P = 0.037). Conclusions: No-tip bacteria were absent in C and present in MR and DS; this result indicates different immune and ecological conditions among these human groups. The score of *Streptococcus mutans* in saliva was higher in DS and MR than in C.

Key words: *Streptococcus mutans group, bacteria score, serotyping, Down syndrome, mental retardation.*

Introduction

Down syndrome (trisomy of chromosome 21, DS) patients show deficiencies, disharmonic and delay of development in relation to the normal child. This affects specially to the immune system, both in its innate or adaptive response, producing a higher susceptibility to infections (1). The most frequent oral pathology present in DS is: permanent open mouth (61%), labial fissure (56%), protrusion of the tongue (42%), macroglosia (43%), striate tongue (61%), pointed arch palate (67%) and irregular alignment of teeth (71%) (1). These conditions determine a special oral ecosystem different from that of healthy individuals. In Chile the most frequent health problems of DS are abnormal dental positions with delay in the teeth eruption (59%) and prognatism (39.2%) (1).

Among the most frequent oral infectious pathologies we found the periodontal disease and caries. The prevalence and incidence of these diseases, in the underdeveloped countries, are associated to their development level and their oral health policies (1-4). In relation to dental caries, the *Streptococcus mutans* group that includes to *Streptococcus mutans* species has been found as and infectious agent (5). For its identification biochemical, serological and genetic (DNA homology) analyses have been used, and in the last years, the 16S ribosomal RNA sequence analysis was added (6). In Chilean children and adolescents (5-19 years old) we found a higher concentration of *S. mutans* and *Candida albicans*, in saliva, among individuals with DS or mental retardation (MR) than in controls (7).

Eight serotypes have been described according to the wall polysaccharides of the *Streptococcus mutans* group : *S.*

mutans (genetic group I with serotype c, e, f), *S. sobrinus* (genetic group III, serotype d, g), *S. cricetus* (genetic group IV, serotype a), *S. rattus* (genetic group II, serotype b), *S. ferus* (serotype c), *S. macacae* (serotype c), and *S. downei* (serotype h) (8,9). The most frequent species in humans are *S. mutans* (c, e, f) and *S. sobrinus* (d, g, h). After the present study a new serotype was identified, serotype k, with a high hydrophobicity, metabolic saccharose dependence for its adherence and low susceptibility to phagocytosis (9). The aim of this study was to identify and quantify with serological methods the *Streptococcus mutans* group in saliva of DS and MR patients of the South Metropolitan Region of Santiago of Chile.

Materials and Methods

Subjects : During 2000 and 2001, 179 male and female children and adolescents, aged 5 to 19 years, were studied. They belonged to the public schools of the Southern Metropolitan Region of Santiago of Chile. Among them 59 presented Down Syndrome (DS), 60 mental retardation (MR) and 60 were healthy control (C) individuals.

Saliva sample: Saliva was collected 2 hours after breakfast. Before the collection a teeth brushing for 30 seconds was performed by a dentist. The center where these children and adolescents are attended gives breakfast daily when they arrive in the morning. Salivary flux was stimulated by applying a 1% citric acid solution in the dorsal face of the tongue. After 1 minute of stimulation, samples were collected by using a sterilized glass funnel and kept at 0ºC for the microbiological analysis. The minimal collected volume was 0.5 ml.

Microbiological Samples: Saliva was homogenized in a Vortex homogenizer (Max MixIItipo 37600 Mixer EE.UU) for 60 seg., then, 100 µL of saliva were mixed with 900 µL of a Na2HPO4 0,2 M (pH 7,4) buffer solution (Sigma, St. Luis, MI, EE. UU). This solution was sonicated for 2 min. at 37ºC and 100 µL were streaked in an TYCSB agar plate [15gr Caseina, 5 g of Yeast extract, 0,2 g L Cystina, (Difco, Detroit. MI, EE. UU), 0,1g Na2 SO3, 1 g NaCl, 2 g Na2 HPO4 x 12H2O, 2 g NaHCO3, 20 g Sodium Acetate x 3H2O, 50 g Sucrose, 15 g Agar for 1 L of water and supplemented with 0,2 U Bacitracine for mL of solution (Sigma, St. Luis, MI, EE. UU)] (10).

Plates were incubated in an anaerobic system (Gas Pack jars with atmosphere generating system 95% N2 and 5% CO2, Oxoid, EEUU) for 48 h at 37 C°. Colonies were counted according to the Westergreen y Krasse's method and scored as forming colonies units per saliva ml. (CFU/ml) (11). Adherent colonies of *Streptococcus mutans* were observed by trans-illumination with the magnifier lens of Spencer EE.UU. (10x).

Biochemical study. The biochemical identification of *S. mutans* or *S. sobrinus* was done by inoculating 2 colonies in the Todd Hewitt broth [3.1g of Brain Heart Infusion, 20g Peptone, 2g Glucose, 2g NaCl, 0.4g Na2HPO4, 2.5g Na2CO3] /L during 18 hours. Bacteria were collected by centrifugation at 5,000 rpm. for 5 min. The pellet was resuspended in 0,2 M Na2HPO4 (pH 7,4) buffer at N° 5 Mac Farland units (1.5x109 CFU/ml). (11).

Serological study. It was performed by the Ouchterlony double immuno-diffusion method (11). Antisera were obtained from female rabbits immunized with *S. mutans* (Ingbritt, serotype c) or *S. sobrinus* (OMZ 176 serotype d), strains were kindly given by professors Bratthall (Sweden) and Loesche (EE.UU). The antigen was extracted by heat at 60ºC during 30 min.

Statistical analysis. The logarithmic score of the number of colonies was studied according to the experimental group, sex, age category and serotype by means of an ANOVA of one and several ways. The homogeneity of the distribution of individuals in categories was performed by a Chi-square test for homogeneity; but for classes with less than 5 individuals the likelihood Chi-square was applied (12).

Results

Tables 1A and 1B show the mean and standard deviation of the bacteria score according to their serotype, experimental Group (C, DS and MR) and age group of males and females.

The most conspicuous result was that in male and female controls (C) no No-tip (no-typed by the used antisera) bacteria were found. No-tip were only found in DS and MR. This finding was significant for the total sample and for male children and adolescents ($p = 0.0025$ and $p=0.026$, respectively), but for females ($p=0.102$).

The ANOVA of the bacteria score was performed in males separated from females: in males the experimental group was highly significant ($p=0.001$), due to a lower score (4.1) among C than among DS (4.2) and MR (5.2), but the difference was mostly given by the high score of MR. When age and serotype were analyzed apart they did not resulted significan, but the group-age interaction resulted significant ($p=0.003$), because C had a higher score at 10-14 years of age than at 5-9 or 15-19 years; while DS and MR increase their score with age. Females did not show significant heterogeneities by Serotype, Group and Age, but they presented a significant Group-Age interaction, because of the same distribution found in males, with the exception that in C the score was similar in the three age categories. It should be remarked that in both sexes the score is lower at 10-14 years of age. The ANOVA for the total sample showed that C had significantly lower scores than MR and DS ($p=0.001$); the Serotype was also significant ($p=0.049$) due to a lower scores of No-tip, with a significant heterogeneity of the score by Serotype and Group ($p=0.035$). There was a tendency to increase the score with age ($p=0.016$), similarly in males and females; but with a higher significance of the Group-Age interaction ($p<0.001$) due to the heterogeneity found in both sexes.

Table 1. A. Distribution of means of bacteria score according to age, serotypes, Nº of individuals and group.

SEROTYPE MALES													
Groups		**c,e,f**			**d,g,h**			**No tip**			**Total**		
	Edad	**Nº**	$\bar{X}$	**DT**	**Nº**	$\bar{X}$	**DT**	**Nº**	$\bar{X}$	**DT**	**Nº**	$\bar{X}$	**DT**
C	**5 a 9**	8	3,4	0,26	2	3,5	0,28	0	-	-	10	3,4	0,26
	10 a14	10	5,0	0,95	0	-	-	0	-	-	10	5,0	0,95
	15 a19	9	4,2	1,42	1	2.0	-	0	-	-	10	3.9	1,50
	Total	27	4,2	1,19	3	3,0	0,89	0	-	-	30	4,1	1,21
DS	**5 a 9**	8	4,1	2,17	1	6,5	-	0	-	-	9	4,4	2,19
	10 a14	6	2,6	0,51	0	-	-	4	3,5	1,77	10	2,9	1,18
	15 a19	6	4,9	0,59	1	6,8	-	3	5,4	1,51	10	5,3	1,03
	Total	20	3,9	1,67	2	6,7	0,21	7	4,3	1,85	29	4,2	1,78
MR	**5 a 9**	8	4,2	1,99	0	-	-	2	2,7	0,92	10	3,9	1,89
	10 a14	8	5,7	1,27	0	-	-	2	5,8	1,41	10	5,8	1,22
	15 a19	8	5,8	0,92	2	6,3	0,45	0	-	-	10	5,9	0,86
	Total	24	5,2	1,60	2	6,3	0,45	4	4,2	2,09	30	5,2	1,64

Group C = Controls; DS = Down syndrome; MR = mental retardation, N = Nª of individuals; $\bar{X}$ = mean[log(streptococci mutans/ml. Saliva)]; DT= Standard deviation; No Tip = no typificable.

Table 1. B. Distribution of means of bacteria score according to age, serotypes, Nº of individuals and group.

SEROTYPE FEMALES													
Groups		**c,e,f**			**d,g,h**			**No tip**			**Total**		
	Edad	**Nº**	- X	**DT**	**Nº**	- X	**DT**	**Nº**	- X	**DT**	**Nº**	- X	**DT**
C	**5 a 9**	9	3,9	0,62	1	3,3	-	0	-	-	10	3,8	0,61
	10 a14	9	5,1	1,21	1	6,1	-	0	-	-	10	3,9	1,31
	15 a19	10	3,9	1,31	0	-	-	0	-	-	10	3,9	1,31
	Total	28	4,2	1,20	2	4,7	1,96	0	-	-	30	4,3	1,22
DS	**5 a 9**	9	4,2	1,42	1	7,0	-	0	-	-	10	4,5	1,60
	10 a14	9	3,9	2,01	0	-	-	1	2,0	-	10	3,7	1,99
	15 a19	7	5,1	1,26	1	6,0	-	2	5,0	1,41	10	5,1	1,41
	Total	25	4,3	1,63	2	6,5	0,68	3	4.0	2.00	30	4,4	1,67
MR	**5 a 9**	9	3,0	1,36	0	-	-	1	2.0	-	10	2,9	1,32
	10 a14	9	4,7	1,55	0	-	-	1	4.0	-	10	4,6	1,47
	15 a19	10	5,7	0,87	0	-	-	0	-	-	10	5,7	0,87
	Total	28	4,5	1,67	0	-	-	2	3.0	1,41	30	4,4	1,68

Group C = Controls; DS = Down syndrome; MR = mental retardation, N = Nª of individuals; $\bar{X}$ = mean[log(streptococci mutans/ml. Saliva)]; DT= Standard deviation; No Tip = no typificable.

Discusion

Control (C) individuals did not present *S. mutans* No-tip, that were found only in DS and MR individuals; however, this type of bacteria could be found in a larger sample of C. The high significance indicates that No-tip bacteria may colonize the oral cavity of C with lower probability than it may colonize that of DS and MR. *S. mutans* No-tip may be another type or to the recent described serotype k, which presents a negative reaction with serotypes c, e and f, due to the loss of the sero-specific glucose united to ramnosa polysaccharides, that were demonstrated by PCR molecular techniques (9). The presence of No-tip strains among DS and MR may be due to particular immune traits or to some oral physicochemical changes (hydrophobicity or hydrophilicity) of these persons. The relative specific and unspecific immunodeficiency of DS individuals may allow these low virulent bacteria for C individuals, with a marginal advantage over other bacteria groups, may compete and stay in the oral cavity; this advantage may be a higher resistance of the k strain to phagocytosis. The high hydrophobicity of the k strain may correlate positively with a higher hydrophobic environment of the oral cavity

of DS and MR. Also, it has been found that the necessary initial adherence of bacteria to development the biofilm is lower in hydrophobic environments (13). The production of hydrophilic saliva peptides by enzymatic breakdown of saliva proteins performed by oral bacteria enzymes enhance the *Streptococcus* growth, which is not favored by hydrophobic peptides (14). Alternatively, the colonization of the biofilm by *Streptococcus mutans* No-tip may be facilitated by other mechanisms as the enzymatic process of the glucosil-transferase (15). However, this is not valid for MR individuals. Perhaps, a denser colonization of the oral cavity changes the ecological parameters of the oral system, leading to changes in the spectrum of primary colonizer strains and species. MR associates to several metabolic diseases that change the conditions of the oral ecosystem. We do not have a satisfactory explanation for the similarity of DS and MR. It is probable that MR and DS have a higher risk to be colonized by No-tip bacteria. This colonization may be a disadvantage because the k-stain associates positively with bacterial endocarditis, produced by the invasion of the blood stream by oral bacteria at the time of an oral intervention (6, 16).

Recent studies have shown that DS individuals have higher levels of the super-oxide-dismutase (SOD) enzyme, encoded in the chromosome 21. This enzyme catalyzes reactions were oxidant radicals are produced; these radicals damage proteins and DNA. The damage could produce conformational changes in salivary proteins and alter the gingival fluid, leading to facilitate the bacterial adherence at the *Streptococcus* No-tip in DS (17).

Controls (C) presented a lower bacteria score than DS and MR for sexes, serotypes and age classes. The higher score of *S. mutans* found in DS and MR agrees with the higher incidence of caries in these groups (1-4). The deficient hygiene in DS and MR could lead to a wider colonization of the biofilm by these bacteria (2, 3). The poor oral conditions in these human groups imply the need for a preventive program of oral health among these patients at high risk of oral pathology. A preventive program of oral pathology has decreased the incidence of caries and periodontal diseases in these groups (2).

The higher score found as the age increases is a well known fact, as well as its relation with sex (non-significant in the present study). The score-age relationship was heterogeneous among groups; it was significant in DS and MR, but not in C, among whom, both in males and females, the score increases in children but decreases in adolescents. This different behavior indicates that the maturation of the oral conditions among these groups could be different in the studied ages.

References

1. Linossier A, Valenzuela C. Streptococcus mutans and Candida albicans in Oral Cavity: Possible Relationship to Down`s Syndrome. In: Malard J editor. Focus on Down Syndrome Research. New York: Nova Science; 2004. p. 213-35.
2. Gabre P, Martinsson T, Gahnberg L. Longitudinal study of dental caries, tooth mortality and interproximal bone loss in adults with intellectual disability. Eur J Oral Sci. 2001 Feb;109(1):20-6.
3. Gizani S, Declerck D, Vinckier F, Martens L, Marks L, Goffin G. Oral health condition of 12-year-old handicapped children in Flanders (Belgium). Community Dent Oral Epidemiol. 1997 Oct;25(5):352-7.
4. Dávila ME, Gil M, Daza D, Bullones X, Ugel E. Dental caries amongst mentally retarded people and those suffering from Down's syndrome. Rev Salud Publica (Bogota). 2006 Sep-Dec;8(3):207-13.
5. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev. 1986 Dec;50(4):353-80.
6. Nomura R, Nakano K, Nemoto H, Fujita K, Inagaki S, Takahashi T, et al. Isolation and characterization of Streptococcus mutans in heart valve and dental plaque specimens from a patient with infective endocarditis. J Med Microbiol. 2006 Aug;55(Pt 8):1135-40.
7. Linossier A, Vargas A, Villegas R, Chimenos E. Quantitative relationship between salivary level of Streptococcus mutans and Candida albicans in children with Down's syndrome. Med Oral. 2002 Jul-Oct;7(4):284-92.
8. Bruckner DA, Colonna P. Nomenclature for aerobic and facultative bacteria. Clin Infect Dis. 1997 Jul;25(1):1-10.
9. Nakano K, Nomura R, Nakagawa I, Hamada S, Ooshima T. Demonstration of Streptococcus mutans with a cell wall polysaccharide specific to a new serotype, k, in the human oral cavity. J Clin Microbiol. 2004 Jan;42(1):198-202.
10. Van Palenstein Helderman WH, Ijsseldijk M, Huis in 't Veld JH. A selective medium for the two major subgroups of the bacterium Streptococcus mutans isolated from human dental plaque and saliva. Arch Oral Biol. 1983;28(7):599-603.
11. Linossier A, Vargas A, Zillmann G, Arriagada M, Rojas R, Villegas R. Streptococci mutans: a semi-quantitative method to assess the risk to oral infection in preschool Chilean children. Rev Med Chil. 2003 Apr;131(4):412-8.
12. Howell D. C. Statistical Methods for Psychology. 3th ed. Pacific Grove C: Duxbury; 2002.
13. Busscher H, Van Der Mei H C. Community structure and co-operation in biofilms.In: Allison D G, Gibert p, Lappin-scott H M, Wilson M. editors.Initial microbial adhesion events: Mechanisms and implications. Great Britain: Cambridge University press; 2001. p. 25-36.
14. Scannapieco F.Oral microbiology and immunology.In:Lamont R, Burne R, Lantz M, Leblanc D, editors.The oral environment. Washington:ASM Press; 2006. p. 47-71.
15. Nakano K, Tsuji M, Nishimura K, Nomura R, Ooshima T. Contribution of cell surface protein antigen PAc of Streptococcus mutans to bacteremia. Microbes Infect. 2006 Jan;8(1):114-21.
16. Takai S, Kuriyama T, Yanagisawa M, Nakagawa K, Karasawa T. Incidence and bacteriology of bacteremia associated with various oral and maxillofacial surgical procedures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005 Mar;99(3):292-8.
17. Zitnanová I, Korytár P, Sobotová H, Horáková L, Sustrová M, Pueschel S, et al. Markers of oxidative stress in children with Down syndrome. Clin Chem Lab Med. 2006;44(3):306-10.

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