COMPARATIVE STUDY OF PREVENTIVE PROTOCOLS IN CHILDREN AT HIGH CARIOGENIC RISK

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ABSTRACT

The aim of this study was to compare the efficacy of two preventive protocols - fluoride gel (F) alone or combined with chlorhexidine varnishes (CHX) - on sialochemical, clinical and microbiological parameters in a group of children at high cariogenic risk.

Two therapeutic-preventive protocols were applied in 73 children at high cariogenic risk (average age 6.2±1.4 years old) and clinical parameters (simplified oral hygiene index: OHI-S; decayed, missing and filled teeth: dmf index; sugar intake and exposure to fluoride), as well as sialochemical parameters (salivary pH and flow, buffer capacity) and microbiological parameters (CFU/mg of dental biofilm of Streptococcus mutans group) were recorded and correlated before and after the protocols. Association was found between parameters that cause deficient control of dental biofilm: high values of OHIS index, CFU/mg dental biofilm, sugar intake and the d component of dmft index, and lower values of salivary flow rate and buffer capacity. After the protocols, a significant decrease was found in OHI-S and CFU/mg dental biofilm. No significant difference was found with children's gender and age.

The association observed between OHI-S and cariogenic bacteria emphasizes the importance of prevention, especially regarding the oral health of the most vulnerable children. The early inclusion of F associated with CHX in the initial step of preventive and therapeutic protocols would provide benefits regarding oral microbe control while children acquire new habits of oral hygiene.

Key words: *dental caries susceptibility, children, saliva, fluoride, chlorhexidine.*

ESTUDIO COMPARATIVO DE PROTOCOLOS PREVENTIVOS EN NIÑOS DE ALTO RIESGO CARIOGÉNICO

RESUMEN

El objetivo de este trabajo fue evaluar la eficacia de dos protocolos preventivos - fluoruro (F) sólo o combinando con clorhexidina (CHX)- sobre indicadores clínicos, sialoquímicos y microbiológicos, en una población de niños de alto riesgo cariogénico.

Se aplicaron dos protocolos terapéutico-preventivos en 73 niños en edad escolar de alto riesgo cariogénico y se determinaron y correlacionaron parámetros clínicos (índice de higiene oral simplificado IHO-S, ceo-d, consumo de azúcar y exposición a fluoruros), sialoquímicos (pH y flujo salival, capacidad amortiguadora) y microbiológicos (UFC/mg de biofilm dental de Streptococcus grupo mutans) antes y después de la aplicación de los tratamientos.

Se observó una asociación entre los parámetros que producen una deficiente control de placa bacteriana: altos valores de IHO-S, de UFC/mg biofilm dental, de consumo de azúcar, del componente c del índice ceo-d y los menores valores de flujo salival y de capacidad amortiguadora. Luego de la aplicación de los tratamientos, se observó una disminución significativa de IHO-S y UFC/mg biofilm dental. No se observaron diferencias significativas con el género y la edad de los niños.

La asociación observada entre los niveles de higiene oral y de bacterias cariogénicas enfatiza la importancia de la prevención y atención de la salud de los niños más vulnerables. La incorporación del F asociada a la CHX en la etapa inicial de los protocolos terapéutico-preventivos ofrecería tempranamente beneficios en el control microbiano mientras se incorporan hábitos de higiene oral.

Palabras clave: *niños, susceptibilidad a caries dental, saliva, clorhexidina, fluoruro.*

INTRODUCTION

Knowledge which has come to light in recent years regarding dental caries has led to a new paradigm and comprehensive approach to its treatment. The multifactorial etiology and complex pathogenesis of dental caries involve microbiological, histological, immune and biochemical aspects, which lead to a diagnostic criterion and therapeutic reformulation for a preventive approach. In odontopediatric clinical practices, it is essential to unify the criteria for a certain diagnosis, identifying individual children or groups of more vulnerable children. With this purpose, clinical indexes have been associated to sialochemical and microbiological tests[1]. The former arise from dental medical

history, both personal and family histories, cultural and socio-economic context, and clinical dental-stomatological examination. Among sialochemical tests, salivary buffer capacity, pH and flow rate are considered as risk factors; while microbiological tests refer to colony-forming units and species identification[2,3].

Considering these indicators, a patient can be categorized as being at high or low cariogenic risk, based on which it is possible to devise preventive and therapeutic strategies tending towards the control each of the factors involved. It is therefore essential to plan preventive actions according to the needs of each group of children, thus reducing costs and improving efficacy[1,4].

In general, preventive actions in children at high cariogenic risk tend to improve host resistance, control cariogenic food intake and, above all, control the pathogenic microbes[5]. The two latter can be managed by changing eating habits and oral hygiene. Depending on their lifestyle and family environment, children may take some time to assimilate these behavioral changes, which require intensive interaction among the child, his/her family and health care professional. While these changes are being implemented, one therapeutic action to control dental biofilm is the use of a chemical that could affect adhesion, colonization and/or bacterial metabolism, such as fluorides (F)[6] and chlorhexidine (CHX)[7]. F has an important inhibitory action on microbial biofilm, conditioning the necessary bacterial metabolic processes for the development and maturation of dental biofilm through enzymatic inhibition[4]. CHX controls the microbiological load in patients, in particular reducing salivary counts for *Streptococcus mutans*, which is highly sensitive to it[8,9].

The aim of this study was to assess and compare the efficacy of two preventive protocols, one applying only F and the other combining F with CHX, on sialochemical, microbiological and clinical parameters and indexes, in a population of children at high cariogenic risk.

MATERIAL AND METHODS

The study was a randomized clinical trial on children with clinical diagnosis of high cariogenic risk (n=73, both sexes, average age 6.2±1.4 years old), who received care at the Department of Pediatric Dentistry, School of Dentistry, National University of Cordoba, Argentina. The study was approved by the Ethics Committee, School of Medical Sciences, National University of Cordoba, Argentina, and parents/guardians of all subjects signed informed consent after a detailed explanation of the protocol. No patient had any history of systemic illness, and all underwent standardized detailed examination of the oral cavity. High cariogenic risk criteria were established by means of the following clinical indicators: dental caries lesions over the past year including white spot lesions, presence of dental pits and fissures, Greene and Vermillion's oral hygiene index simplified[10] (OHI-S) ≥ 0.8, sugar intake > 4 per day and low frequency of visits to the dentist[11]. The following parameters were determined in this high caries risk group: *Microbiological parameters*: counts of *S. mutans* group in dental biofilm collected from the lingual face of mandibular molars with a periodontal probe, which was immediately placed in sterile physiological solution in pre-weighed tubes. Samples were immediately cultivated (mg of dental biofilm collected) in duplicate, in mitis salivarius agar with bacitracin (DIFCO®, Becton Dickinson, France), incubated for 48 hr at 37ºC in microaerophilic environment; colonies were counted (CFU/mg of dental biofilm). Biochemical identification was performed by means of morphological characteristics and conventional biochemical tests (Diatabs®, Doughnut Diagnoses, Denmark)[12]. *Sialochemical parameters*: Saliva samples (stimulated by chewing a piece of 3x3 cm2 paraffin film) were collected by direct salivation between 9 and 11 a.m. for 5 min in iced graduated tubes. Patients had been advised not to eat anything for at least 2 hours before the sample collection. Salivary flow rate, buffer capacity and pH were determined immediately (Orion® pH meter, USA)[2,3,13].

All children received initial preventive therapy consisting of oral hygiene techniques, dietary advice and caries inactivation. After that, they were randomly divided into two groups for the application of preventive protocols under evaluation: Group I: (n=36) received a weekly topical application of fluoride gel for four weeks (F: acidulated gel 1.23% NaF, Gelato, Deepak, USA), and Group II: (n=37) received combined therapy, alternating topical acidulated fluoride application with 1% chlorhexidine varnish, for the same period and frequency (F + CHX: Periobacter Prof, NAF, Argentina). Clinical, sialochemical and microbiological parameters were assessed again one week after the final application of the different treatments. The analysis of correspondence association required operationalization of variables and determination of levels, considering the median for the quantitative variables: level 1: lower or equal to the

median; level 2: above the statistical median. For OHI-S, ≥ 0.8 values were included in level 2, while level 1 corresponded to < 0.8 values.

Statistical Analysis

Categorical variables were summarized as proportions, and comparisons of groups were made by Student's *t* test for paired samples, ANOVA and correspondence analysis, considering a significance level of p<0.05 (Infostat Professional, version 2007).

RESULTS

The sample was made up of 61.8% female and 39.2% male participants. Clinical, sialochemical and microbiological variables at baseline (pretreatment) are shown in Table 1.

Association between the parameters studied and clinical indexes before the application of the treatment was carried out by multivariate correspondence analysis. Association was found between deficient levels of parameters that control cariogenic dental biofilm: high values for OHI-S, CFU/mg of dental biofilm, and sugar intake, and poor buffer capacity; as well as high levels of active caries with the *d* component of the *dmf-t* index and lower values of salivary flow rate. Association was also found between lower levels of OHI-S and CFU/mg of dental biofilm and better buffer capacity. Lower levels of variable active caries, the *d* component of the *dmf-t* index and higher sugar intake values were associated with higher values of salivary flow rate (inertia 37.85%). No association was observed between salivary pH and children's gender; nevertheless, lower buffer capacity was found in female patients. Regarding children's age, a tendency towards lower salivary flow rate was observed in younger children (≤ 5 years old, p=0.08), probably related to the difficulty in salivating and collecting saliva.

After both treatments, the levels of CFU/mg dental biofilm decreased significantly in relation to pretreatment values (F: p=0.004; F + CHX: p=0.001). Comparing both treatments, the decrease in CFU/mg dental biofilm was greater in Group II (F + CHX treatment) (p <0.0001) (Fig. 1). Moreover, OHI-S decreased significantly with treatment (treatment F: p=0.0002, treatment F + CHX: p<0.0001) (Fig. 2). No significant difference was found between treatments. Higher values of CFU and OHI-S were observed in children over 6 years old, with more significant differences. No significant difference was found between girls and boys.

Table 1: Clinical, sialochemical and microbiological parameters of children before treatments.

		Pre- Treatment Values
Clinical parameters	*dmf-t*	5.2 ± 2.4
	d component	3.7 ± 2.3
	f component	0.9 ± 1.4
	OHI-S	1.5 ± 0.5
	Moments of sugar per day	4.5 ± 1.5
Sialochemistry and microbiological parameters	Salivary flow	0.78 ± 0.36
	Salivary pH	7.51 ± 0.35
	Salivary buffer capacity	5.92 ± 0.67
	CFU / mg biofilm	(2.06 ± 3.80) x 10^8

Data are expressed as mean ± standard deviation

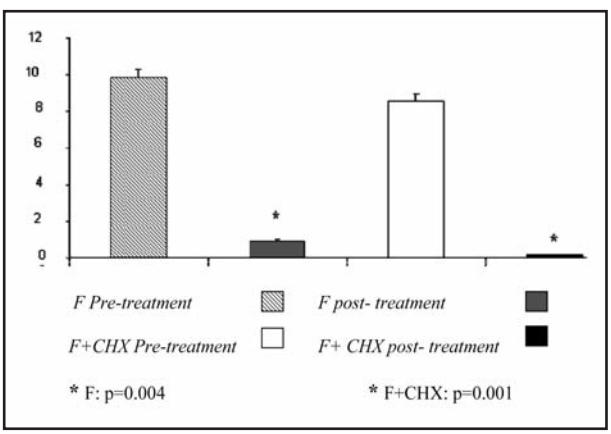

Fig. 1: Effect of therapeutic preventive protocol treatment on S. mutans levels (CFU/mg biofilm).

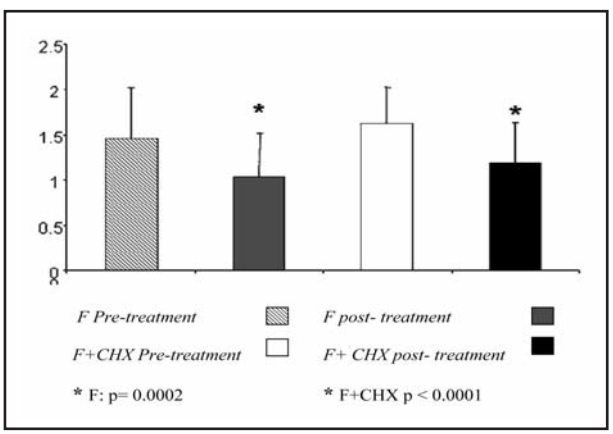

Fig. 2: Effect of therapeutic preventive protocol treatment on the oral hygiene index (OHI-S).

DISCUSSION

Although there is much research on cariogenic risk employing clinical, sialochemical and microbiological parameters as risk indicators at baseline and after treatment, there is very little information about young school-age population in our region. The high prevalence of caries among children warrants further research on the subject, which encouraged us to contribute this study. Clinical, microbiological and sialochemical variables were selected from bibliographic reviews[2,8,11,13] and our clinical experience allows us to establish points of reference. Although there are many indexes for quantifying the presence of oral biofilm, in odontopediatric practices, the Green and Vermillion Simplified allows biofilm in primary teeth to be measured quickly and easily, and to be associated to oral hygiene status [10].

Like other researchers, we have established assessment protocols and treatments for children at high cariogenic risk adapted to our context. Such protocols are based on oral hygiene, history of dental caries, dietary habits, state of oral health, oral microbial load, salivary flow rate and pH[13]. In this regard, Zero et al.[14] found that the model for the assessment of risk factors depends on the population studied; Peterson et al.[15] reinforces this idea, suggesting that geographical area can be considered as a predictor, as well as social factors, lifestyles, and cultural and economical aspects.

Many protocolized clinical trials associate the promotion and education of oral health with the application of chemical substances, combining mainly fluoride and chlorhexidine[7,16,17], as well as studying the relationship between *S. mutans* levels and different stages of early childhood caries[18]. Nevertheless, and in agreement with Rijkmo et al.[19], it is difficult to compare results because of the diversity of methodological conditions and heterogeneity of populations. This is the case for *S. mutans* counts, reported in our study as CFU/mg dental biofilm, which are not comparable with some published values, usually reported as CFU/mL saliva[5,8,12]. In this study we have considered the fact that CFU associated to dental biofilm are much more closely related to cariogenic risk than are planktonic bacteria in saliva.

Our study found an improvement in the level of OHI-S and CFU/mg dental biofilm after treatments with more statistically significant levels when the sample was stratified according to age, which could be related to a greater efficacy of educational protocols and greater motor ability in the older group of children.

Regarding the observed *dmf-t* index values, the high prevalence of the *d* component observed with a low level of the *f* component indicates the lack of attention to oral health among these children; moreover, high values of the *d* component determine, together with other parameters, a future prognosis of high caries risk[20,21]. These authors noted how powerful the *d* component is as a predictor of caries on permanent dentition and therefore, how important educational programs to recover oral health are.

In our study, salivary flow rate values corresponded to high and medium risk values according to Bordoni et al.[22], medium risk values observed by Sánchez-Pérez et al.[23] and were lower than those reported by Tukia-Humala et al.[24]. These findings reinforce the need for local studies describing the special characteristics of vulnerable children in our region.

The salivary buffer capacity values observed in our study did not correlate with those reported in other papers according to caries risk and age[25]. These papers found that salivary buffer capacity and flow rate improved after tooth brushing techniques were taught, emphasizing the importance of implementing preventive and educational programs. Our results showed higher values of salivary flow rate than those reported in the literature according to the cariogenic risk observed[22,26].

The decrease in *S. mutans* counts and improvement in oral hygiene index after fluoride application combined with CHX varnishes, added to the educational motivation and preventive and educational actions, were similar to those reported by Petersson et al.[15]. Many authors[26,27] confirmed the benefits of applying chemical compounds in order to reduce the bacterial load and control caries, emphasizing the benefits of the application of fluoride combined with CHX varnishes in patients at high cariogenic risk.

Twetman[28] showed the antimicrobial properties of CHX, although its effectiveness on long-term prevention was not proved. Experimental studies with both antimicrobial agents also showed a greater effectiveness in the prevention of biofilm development, and thus of enamel demineralization[29]. The association observed between oral hygiene index and cariogenic bacteria counts in this study supports the need to emphasize preventive actions for maintaining oral health in the most vulnerable children; this could also demonstrate that OHI-S reflects pathogenic *Streptococcus* levels, although it must be remembered that dental caries is a multifactorial disease, and other factors such as oral hygiene and access to fluoridated products are also determinants of the carious process. In this sense, prevention should be based on diet control, teaching oral hygiene techniques and reinforcing

host resistance. Nevertheless, including CHX during the initial phase of clinical odontopediatric actions in children at high cariogenic risk offers benefits in microbial control while dietary and oral hygiene habits are being promoted. This study shows that the application of CHX together with fluoride varnishes is an important complement to initial therapy in children at high cariogenic risk and provides better control of some indicators than does using only fluoride. Nevertheless, and considering that our work cannot be interpreted as an epidemiological study, further studies are needed to assess the efficacy on long-term prevention.

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