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Control of Dental Biofilm and Oral Health Maintenance in Patients with Down Syndrome

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<http://dx.doi.org/10.5772/53348>

1. Introduction

The greatest prophylaxis challenge in dentistry is the control of dental biofilm and consequently, avoiding dental caries and gingival inflammation [1]. This control is generally carried out through mechanical and/or chemical methods. Although the mechanical methods (toothbrush and dental floss) are considered efficient, they are not sufficiently so in certain cases [2], [3].

Individuals with Down syndrome (DS) present various oral diseases, such as the presence of pseudoprognathism, hard palate and lower ogival shape; pseudomacroglossia due to hypotonia tongue; high prevalence and susceptibility to periodontal problems due to error in the autoregulatory mechanism immune, and poor occlusal relationship, with a predominance of anterior crossbite and / or later. The position of the tongue protruded, produces abnormal strength in the lower anterior teeth, which normally are in a position to cross-bite. These factors favor the onset of severe periodontitis, leading to early loss of teeth. However, there is a lower incidence of dental caries, which has been attributed mainly to the increase in buffer capacity of saliva [4].

Some dental anomalies can be observed, as the presence of hypodontia or oligodontia, tooth conoids, microteeth, hypocalcification enamel, fusion and twinning can also be an increase in the size of the clinical crown of molars and the inclination of the occlusal surface to the lingual, making access to restorative procedures. Furthermore, the rash and exfoliation of the primary teeth and eruption of the permanent are delayed, and there is a high prevalence of bruxism [4], and these alterations interfere with the quality of toothbrushing.



Figure 1. Pseudomacroglossia due to hypotonia tongue

There is agreement among many authors on the existence of factors predisposing to periodontal disease in patients with Down syndrome. Although poor oral hygiene, poor nutrition and local irritants may exacerbate this problem, they can not be regarded as its main cause. The greater predisposition to periodontal disease has been attributed to characteristics of patients with chromosomal abnormalities of trisomy [5] It is therefore essential to establish strategies to prevent periodontal disease in these individuals.

Second Cornejo et al. [6] (1996), which conducted a study in 86 individuals with DS living in Argentina, aged between 3 and 19 years, the presence of the changes described above puts them at a disadvantage in relation to oral health, compared with noncarriers.

Besides the inherent disadvantage to the individual, access to dental care is also difficult for these people. Allison et al. [7] (2000), in a study conducted in France, compared the levels of care received dental services and oral hygiene habits among children with DS and their siblings. According to parents and / or guardians, the group with DS had difficulty finding access to dental services and oral care compared to their phenotypically normal siblings. In Brazil, studying the prevalence of dental caries in primary and permanent teeth of children with DS in Sao Jose dos Campos (SP), Moraes et al. [8] (2002) found that the values of ceo and CPO-D were similar to those identified by the Municipal Health Department, in a survey of dental caries in children from public schools. However, the authors found a frequency of 9.25% and 4.76% decayed teeth restored among the children examined, against the values of 3.98% and 5.88%, respectively, obtained by the Municipal Health.

All these mentioned aspects can be inferred that it would be essential to adopt appropriate measures aimed at controlling biofilm among the DS patients, to prevent the installation of dental caries and gingival inflammation, because the microorganisms in the biofilm and act

decisively etiologic agents in the origin and development of caries and periodontal also (König et al. [9] 2002). In 1965, Loe et al. [10] demonstrated the direct relationship between the biofilm and the development of gingivitis in humans, concluding that the removal of biofilm employing brushing and flossing, could result in reversal in health (Loe et al. [10] 1965, Theilade et al. [11] 1966). For this reason, control of the biofilm has an important role in the prevention, treatment and maintenance of periodontal health.

2. Mechanical control of dental biofilm

The mechanical control is to remove biofilm employing proper technique of brushing, combined with a dentifrice and auxiliary materials such as wire or dental tape (Owens et al. [12] 1997).

The ability to remove dental biofilm by the use of different types of brushes is basically the same. There is no ideal brush, and your choice should be guided by the needs of each individual patient and clinical observations of the professional. However, there are characteristics that facilitate the oral hygiene procedures, as the presence of small head multitufuladas, soft bristle, rounded second study by Panzeri et al. [13] (1993).

The toothbrushing is an effective procedure for the maintenance of proper oral hygiene. However, to get a good cleaning of the oral cavity, in addition to toothbrushes, other factors must be considered such as time, frequency, brushing technique, manual skills and motivation of patients (Halla [14] 1982).



Figure 2. Motivation of patients and toothbrushing

Several authors report that, although brushing is the most widespread and universally suitable for the mechanical removal of the plaque, there are not known techniques ideal nor brushes which, by itself, may promote a perfect cleaning. All this technical device should be associated with constant motivation [12],[15]-[16].



Figure 3. Toothbrushing technique and manual skills

The control of dental biofilm is a preventive action that involves a number of aspects, such as health education, which is achieved through constant guidance and motivation for people on oral hygiene (Bijella [17]1999).

The manual dexterity and, many times, the motivation, are indispensable factors for efficient oral hygiene through mechanical means in patients with Down syndrome [18]-[22]. Thus, the key to success in promoting and maintaining a satisfactory oral health in these patients is the application of a rigorous program of oral hygiene constant [23].



Figure 4. Lecture to motivate the control of biofilm

Mental disability is another aspect to be considered as difficult to awareness of the importance of oral health, difficulty in learning the techniques of brushing and lack of concentration at the time of toothbrushing [24],[25]. This difficulty leads these patients to have high levels of plaque-dependent oral diseases, especially periodontal changes ⁶. It is therefore essential to establish strategies to prevent periodontal disease in these individuals.



Figure 5. Constant guidance and motivation

Second Nielsen [26] (1990), the type and degree of disability are also important factors, since the greater the degree of mental deficiency the worse the level of hygiene.

3. Dentifrices with disclosing agent dental biofilm

The obstacles inherent to children with Down syndrome and the difficulties faced by parents and /or people in charge for toothbrushing, lead the professional in dentistry to look for a substance capable of aiding and stimulating these patients in the mechanical control of the dental biofilm. Studies suggest the use of disclosing agents, such as erythrosine, to remove dental biofilm more easily. For this reason, the presence of a disclosing agent in the formulation of the dentifrice could aid in the removal of the dental biofilm [27].

Are disclosing the chemicals used for staining bacteria, which show the colonies, invisible or barely visible, that adhere to tooth surfaces, making them visible, thus supporting the maintenance of oral hygiene while facilitating their removal (Bellini et al. [27]1974). Among the forms of application of disclosing the most commonly used in dentistry are tablets or solutions (Medeiros [28]1991).

The proven merit of disclosing meant that its use became a source of motivation (Toassi, Petry [29]2002), are indicated as excellent aids in determining the state of oral hygiene. Shown to be valuable as a teaching tool in education, not only by convincing the population for the presence of dental biofilm, as well as raising awareness about the need for its removal (Cristiano, Bignelli [30]1995).

Second Bouquet [31] (1971) and Gillings [32] (1977), the disclosing must provide ease of application and handling, good flavor, not blushing residually plastic restorations or tooth cracks, do not stain the mucosal lip, cheek and gum, to be of contrasting color facilitate the differentiation from the marginal gingiva and does not cause tissue irritation.

There are a variety of disclosing the market, among them are methylene blue, eosin, erythrosin, fluorescein sodium, neutral red and proflavine monosulfate. According to the work of

Silva et al. [33] (2002), among all the solutions mentioned, eosin, erythrosine and neutral red showed the greatest ability to blush, ease of removal and absence of antimicrobial activity, are essential requirements in studies evaluating methods of hygiene and guidance patient.

Erythrosine a dye consisting of the disodium salt of 3', 6' - dihydroxy - 2', 4', 5', 7' - tetraiodo-spiro [isobenzofurano -1 (3H), 9 - [9H] xateno] - 3 - one and may contain up to 4.0% Fluoresceins a lesser degree of iodination, and chloride and / or sodium sulfate and water of crystallization. Must contain at least 85% calculated as total dye $C_{20}H_6I_4O_5Na_2$. Presented as physical characteristics: fine powder, red or brown odorless, soluble in water and hidroscópio giving red solution should not exhibit fluorescent room light, also soluble in ethanol, glycerin and propylene glycol. Practically insoluble in ether, mineral oil and fats (Standing Committee Review of the Brazilian Pharmacopoeia [34] 1996).

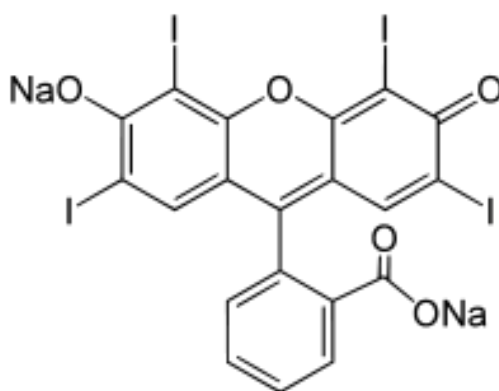


Figure 6. Structural formula erythrosine

The use of a dentifrice containing the color erythrosine as agent for removal of dental biofilm during toothbrushing is an excellent resource to stimulate the patient in your dental hygiene (Quintanilla, Bastos [35]1988), because the presence of this dye to facilitate parents and individuals / or guardians to view the plaque, especially in places where there is greater difficulty of removal during brushing (Duarte et al. [36]1990).

The use of toothpaste containing erythrosine, Dentplaque ®, was approved by the ADA, and is used as part of a program to promote oral health, being distributed by the Ministry of Health in 1999, the Health Secretariat of São Paul, including the Regional Health of Piracicaba, Piracicaba encompassing than 25 cities in the region (Silva et al. [37] 2004).

According to research Quintanilla et al. [38] (1989) where they studied the clinical behavior of the dentifrice added erythrosine Dentplaque ® 0.5% by comparing the new proposal to existing, as the common dentifrice and dental plaque disclosing in tablet form coadjutor dentifrice common comparing the percentage of plaque remaining and the time taken to perform each of the three proposals in nine females with mean age of 21.33 years, and all with private have never experienced the use of a plaque disclosing. Individuals selected for the sample received no instruction on brushing technique, since the aim was to assess whether humans would be able to remove plaque evident on the surfaces of the teeth, ac-

according to the manual skills of each participant. The time was recorded in seconds since the beginning of each experiment (opening the packages), to its end. To evaluate the remaining plaque, disclosure was made with basic fuchsin after each experiment, and recorded the number of stained surfaces, indicating the remaining plaque. The authors found that the average time of tooth brushing with toothpaste containing erythrosine has become more than double when compared to an ordinary toothpaste. Regarding the plaque index, the authors observed that the impregnation of the dye this is most efficient method III (Dentplaque®), because the dye is rubbed on the plaque while toothbrushing, when compared to other (MI - toothbrushing with dentifrice common; M II - use of disclosing tablets plaque and toothbrushing with dentifrice common).

However, Rodrigues et al. [39] (1994) found different result. Undertook a study on the effectiveness of the dentifrices containing erythrosine Dentplaque® in the stimulation process to dental hygiene for 45 male children, aged 6-12 years living in an orphanage in the city of Rio de Janeiro. These children were divided randomly into three groups of 15 patients maintained the same dietary habits. Initially, all received instructions on oral hygiene and toothbrushing technique, through lectures and posters illustrative devices. Were given tuition every 30 days during the 90 days of the survey. The brushing technique recommended in this study was to headphones, and recommended its implementation soon after meals. The control group made use of the brush with your regular dentifrice, the second group made use of a disclosing in tablet form before each brushing, and use your usual toothpaste, and the third group used a dentifrice containing erythrosine for toothbrushing routine. These children were supervised daily by an official of the orphanage properly oriented. In the initial evaluation, all were subjected to three more evaluations, with a 30-day interval between them being given the simplified oral hygiene index of Greene & Vermillion. The authors concluded that there were no statistical differences in relation to reducing the level of dental plaque in the three groups, but it was observed that the dentifrice was the easiest way of disclosure, and inserts a method of assimilation more difficult for children aged 6-12 years.

The same result of the work of Rodrigues et al. [39] (1994) found in Silva et al. [97] (2004), with 62 students at a public school in the city of Piracicaba, aged between 12 and 14 years. Participants were divided into groups: dentifrice with erythrosine Dentplaque® (Group I) and the use of disclosing tablets (Group II). The plaque reduction was observed in all groups did not show statistically significant differences between them. However, the authors noted that factors that had limited the completion of this study, as the amount of sample, the low amount of plaque revealed by the index and the small amount of plaque shown by the students may have influenced the results, covering the response of the methods. In addition, the fact that some individuals participated in this study only the initial assessment, refusing to participate in the final evaluation, the amount of the sample was reduced to 18 participants.

In this context, the use of a dentifrice with erythrosine, as an agent for plaque removal should encourage the completion of a thorough toothbrushing, presumably more closely individual (Silva et al. [40] 2003).

4. Chemical control of dental biofilm

Studies have shown that mechanical control produces significant reductions in gingivitis in people with special needs. However, many patients with Down syndrome, besides being unable to cooperate, do not have sufficient manual dexterity to do toothbrushing or to use dental floss. Consequently, the use of chemical and / or antimicrobial agents as aids in plaque control can be indicated for these individuals. Considering the fact that toothbrushing with dentifrice is the most common tool for good oral hygiene, adding chlorhexidine to dentifrices could be seen as a practical means of improving the quality of oral hygiene [2, 41-47].

The chemicals and / or antimicrobial agents are often used in dental plaque reduction and can be used in conjunction with the mechanical control in preserving health and treatment of gingivitis, in some patients (Mandel [48] 1994), especially those that have little manual dexterity to the realization of toothbrushing (Fischman [49] 1979).

The attributes required for a chemical agent can play its effectiveness in controlling supragingival biofilm was postulated by Loesche [50] (1976). According to the author, the chemical agent to be effective against microorganisms responsible for inflammation and must have substantivity, ie, the intraoral retention capacity, to achieve a contact time sufficient to act on the microorganisms existing, and to maintain inhibition dental biofilm formation by a longer period. Furthermore, the product must be stable at room temperature for a considerable time and safe for human use.

Other features should also be observed for a chemical agent to be considered effective, such as lack of toxicity, not to be allergenic, have clinical evidence of significant reductions of plaque and gingivitis, be selective and have specificity to act on pathogenic microbiota, provide a pleasant taste have to be affordable and easy to use (Van Der Ouderra [51] 1991).

Chemical control of biofilm can be made to prophylactic or therapeutic. In the first case, the goal would be that there were an imbalance in the microbiota, when mechanical methods are ineffective. In the therapeutic sense with respect to individuals who already have changes in order to achieve the predominant bacteria-related diseases, aiming at restoring the microbiota and its harmony with the host (Marsh [52] 1992).

In 1954, Davies et al. [53] synthesized in the laboratory substance large bacterial action against Gram + and Gram -, and fungi. From this time, the chlorhexidine is now used as a general disinfectant for the treatment of various infections.

It was marketed in the 60s, by Imperial Chemical Industries (England), and one of the first applications of chlorhexidine in dentistry to control biofilm was performed by Schiott and Løe [54] (1970). The authors recommend the use of 10 mL of chlorhexidine digluconate 0.2% twice a day for one minute in order to prevent the accumulation of plaque and gingivitis subsequent. Since then, this compound has been considered the most effective agent in the chemical control dental biofilm (Souza, Abreu [55] 2003).

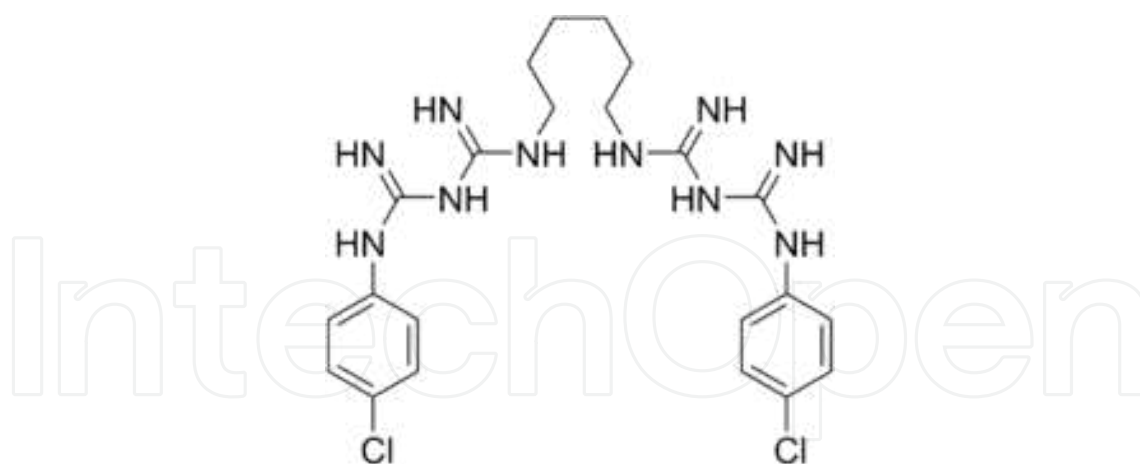


Figure 7. Structural formula chlorhexidine

Chlorhexidine is a cationic agent, a bis-guanidine non-toxic molecule is a symmetrical, with two rings and two 4-chlorophenyl groups ethane pentânicos connected by a central hexamethylene chain. Is prepared in the form of various salts, and gluconate, the digluconate or chlorhexidine acetate in its composition (Vinholis et al. [56] 1996). The chlorhexidine digluconate salt is one of the most widely used in the preparation of therapeutic formulations, because of its greater solubility in water and physiological pH, dissociates releasing the active component (Bonacorsi et al. [57] 2000).

The main site of action of chlorhexidine, both in prokaryotes and in eukaryotic cells is the cytoplasmic membrane. The mechanism of action of chlorhexidine begins with a call in the bacterial cell wall, when the adsorption of positive charges in the molecule of the substance to the surface of the negative charges increases the permeability of the bacterial cell walls of microorganism and allows the agent to penetrate the cytoplasmic occurring disruption of cell membrane leakage of intracellular components and low molecular weight, as potassium ions. At this stage the bacteriostatic effect is considered and reversible. While in high concentrations, lead to enzyme inhibition (ATPase), extravasation of macromolecules (nucleotides) and clotting components of the cytoplasm, due to the interaction of chlorhexidine with cytoplasmic proteins and nucleic acid, thus reaching the stage of bactericidal and irreversible (Bonacorsi et al. [57] 2000).

The chlorhexidine is usually effective against Gram positive and Gram-negative bacteria, fungi, yeasts and *Candida albicans*. It has broad spectrum antibacterial, high substantivity, is safe and effective (Quagliato [58] 1991).

Second Vinholis et al. [56] (1996), there are three mechanisms for chlorhexidine inhibition of biofilm:

Chlorhexidine is connected by means of electrostatic forces to the groups of acidic proteins such as phosphates, sulphates and carboxyl ions found in saliva and mouth tissues, there avoiding the formation of the acquired pellicle.

The ability of bacteria to bind to the tooth can be reduced by the absorption of chlorhexidine to the capsule of extracellular polysaccharides.

Chlorhexidine can compete with Ca^{++} ions. The mechanism is probably due to a direct competition between ions and / or availability of the drug and the carboxylic groups in the oral tissues. Can also inhibit the formation of bridges between the Ca^{++} bacteria and surfaces, and the bacteria together. Due to its cationic properties, chlorhexidine can bind to the hydroxyapatite of enamel, and the acquired pellicle salivary proteins (Gjerme 59 1989).

5. Dentifrices with chlorhexidine

The chemical agent chlorhexidine as deputy in the control of dental biofilm is useful in situations where oral hygiene is inefficient, is compromised or is impossible to be realized. This antimicrobial agent is particularly suited to individuals who, because of physical or mental limitations, they are incapable, in whole or in part, the appropriate mechanical removal of plaque, were considered patients with special needs (Al-Tannir, Goodman [60] 1994).

That the dentifrices are used in conjunction with toothbrushing, causes the addition of chlorhexidine greater deserves attention, since it does not represent changes to the patient, as is routine in the same. Importantly, most studies of dentifrices containing chlorhexidine has been made with experimental formulations (Sathler, Fischer [61] 1996).

Experimental studies have shown that dentifrices with 0.5% chlorhexidine were less effective than rinsing mouthwash with 0.2% chlorhexidine (Addy et al. [62] 1989, Jenkins et al. [63] 1990). In a study by Gjermo and Rolla [64] (1970), the use of dentifrices with 0.6% chlorhexidine and 0.8% applied in trays on the teeth to avoid interference from the mechanical action of toothbrushing, showed a reduction in the rate of plaque, and these results were consistent with those obtained with mouthwash.

Second Jenkins et al. [42] (1993), introduction of 1% chlorhexidine dentifrices promoted to an improvement in gingival index and plaque index, similar to those experienced in rinsing with 0.2% chlorhexidine. The authors also state that the association of fluoride with chlorhexidine dentifrices does not inhibit chlorhexidine.

The use of chlorhexidine dentifrice is a controversial subject. Some research on the short-term clinical effect of reducing plaque and gingival show the effectiveness of this substance (Torres 65 2000). This was proved in the study of Storhaug 46 (1977), which evaluated the use of toothpaste containing 0.8% chlorhexidine in 27 patients with special needs, from 4 to 12 years in a clinic held by the government of Norway. These patients were selected to test the effects of toothbrushing performed with the plaque index, gingival index, according to the criteria proposed by Löe and Silness. Patients were then divided into two groups: 17 children were using toothpaste containing chlorhexidine (GI) and 10 children used a placebo dentifrice (GII). After 6 weeks of study, there was significant reduction in plaque index of the group that used chlorhexidine compared with the control group and gingival index, no

significant differences for the group that used chlorhexidine. However, clinically, the acute signs of inflammation are gone. The author stated that the conventional techniques of oral hygiene can be difficult to implement for this group of patients and chlorhexidine, in its various forms of application, an agent is extremely useful for maintaining oral health of patients with special needs.

Russell and Bay [44] (1981) observed that the use of toothpaste the basis of 1% chlorhexidine in daily brushing of children with epilepsy and mental retardation, reflected in a significant improvement in plaque and gingival index in this group of patients.

Dolles and Gjermo [41] (1980) evaluated the effect of three dentifrices in reducing dental caries and gingivitis (DI - dentifrice containing chlorhexidine (2%), IBD - with fluoride toothpaste (0.1% NaF) and DIII - chlorhexidine dentifrice with the two % and fluorine (0.1% NaF) for two years. Ninety-one students from 13 to 15 years of age participated in the research. the group using the dentifrice with fluoride and chlorhexidine showed a lower rate of dental caries, although the gingival conditions improved in the three groups, showing no statistical differences.

In a study of experimental gingivitis, Jenkins et al. 42 (1993), found that a dentifrice formulation of 1% chlorhexidine and 1000 ppm F (NaF) produced statistically significant reductions in plaque and gingivitis, compared with the placebo dentifrice. Subsequently, Yates et al. 47 1993, proposed to assess the clinical effects of chlorhexidine dentifrice 1%, with or without the 1000ppmF (NaF) previously tested by Jenkins et al. 42 1993. This study aimed to evaluate the control of plaque and gingivitis using: a) dentifrice containing 1% chlorhexidine called single asset, b) 1% of dentifrice containing fluoride clorexidina/1000ppm called active double c) negative control for six months. The sample consisted of two hundred ninety-seven individuals aged between 18 and 61 years. The periodontal parameters used were the plaque index, gingival bleeding and staining that were recorded at the beginning, six, 0,24 weeks, along with the index calculation was also recorded in the sixth, twelfth and twenty-fourth week. After prophylaxis performed at baseline, the subjects used the assigned dentifrice twice a day for one minute, without any other additional information on oral hygiene were given, just the direction we should use enough toothpaste to cover the head of the toothbrush. It was not permitted to use any other adjunctive oral hygiene product. At the end of the study all subjects were examined by a hygienist and extrinsic staining, supragingival plaque and calculus were removed. The results showed reduction of plaque index and bleeding in all groups, but a significant improvement occurred in the chlorhexidine group. In contrast to these results, staining and calculus indices were more significant in the test groups compared with the control group. The authors concluded that the side effects of chlorhexidine are acceptable, the dentifrice containing chlorhexidine can be recommended for the same clinical applications than the other products based on chlorhexidine. The compatibility of fluoride with chlorhexidine in one of the products could be effective in preventing tooth decay, and fluoride dentifrice containing chlorhexidine and could provide benefits to gingival health than preventive and therapeutic applications in clinical dentistry.

The action of a dentifrice containing 1% chlorhexidine in reducing dental plaque and gingival bleeding in 156 children over a period of twelve weeks, residents in Ga-Rankuwa (Pretoria)

ria, South Africa), aged between 12 and 14 years were evaluated by Gugushe et al. [2] (1994). The children were divided into three groups, which used conventional dentifrice (group A - 51 subjects), placebo dentifrice (group B - 49 individuals) and chlorhexidine dentifrice (group C - 56 individuals). Before starting the experiment, they were instructed on oral hygiene, had their records of plaque index, gingival taken and received professional dental prophylaxis. The record of the indices was repeated in the sixth and twelfth weeks. All patients were instructed to make tooth brushing morning and night. In the presence of plaque, it was observed that the rate decreased in all groups, with reductions substantially equal groups A and B and further reduction to the group C. In relation to the gingival index, a reduction very similar in all groups (approximately 4%) without significant differences. However, the dentifrice with 1% chlorhexidine was more effective in controlling dental plaque as compared with the conventional dentifrice and placebo.

In a clinical study by Sanz et al. [45] (1994), the experimental dentifrice containing chlorhexidine 0.4% and 0.345 mg of zinc, contributed significantly to the improvement of oral hygiene, both in relation to the plaque and gingivitis and bleeding, resulting in fewer spots than those found in the group who used mouthwash with chlorhexidine 0.12%. The investigators concluded that the tested dentifrice can be viewed as a promising alternative for the use of substances effective in reducing plaque and gingivitis, and offer minimal side effects.

In respect the effect on the microflora of the mouth, the dentifrices to 1% chlorhexidine and tested for a period of 6 months, promoted reduction of aerobic microorganisms and aneróbicos (Maynard et al. [66] 1993).

Considering the fact that toothbrushing with dentifrice is the most common habits of oral hygiene (Owens et al. [12] 1997), this practice can be seen as a plausible way for the introduction of chemicals to improve the oral health (Yates et al. [47] 1993).

According to Newman [67] (1986), the introduction of antimicrobial agents in dentifrices aims to improve the effectiveness of toothbrushing, promoting a positive effect in reducing biofilm.

Thus, Teltelbaum et al. [68,69] (2009, 2010) conducted a study with patients with SD, where he developed a dentifrice containing these two substances, chlorhexidine and erythrosine and evaluated the mechanical and chemical control of dental biofilm. The mechanical and chemical control of dental biofilm in patients with Down syndrome, of using different experimental dentifrices in forty institutionalized children between ages 7 and 13 years in the mixed dentition in an experimental cross-over, blind clinical trial where we used the following protocols: fluoridated dentifrice (protocol G1); fluoridated dentifrice + chlorhexidine (protocol G2); fluoridated dentifrice + chlorhexidine + plaquedisclosing agent (protocol G3); and fluoridated dentifrice + plaque-disclosing agent (protocol G4). Each experimental stage lasted 10 days with a 15-day washout. The evaluated parameters were plaque index and gingival bleeding and initial clinical conditions between each stage were similar. The dentifrices containing plaque-disclosing agent, irrespective of their association with chlorhexidine, produced a greater reduction in the final plaque index. As for gingival bleeding, the dentifrice containing erythrosine and the one containing chlorhexidine produced similar re-

sults. The dentifrice containing an association of chlorhexidine and erythrosine gave the best results. Thus, with the methodology employed, it was possible to conclude that the combination of drugs (chlorhexidine, fluorine and erythrosine) within one dentifrice can be useful in controlling dental biofilm and in the reduction of gingival bleeding [68,69].

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