



THESIS SUMMARY

Black Stain and Caries in Deciduous and Mixed Dentition.

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INTRODUCTION

The already erupted primary and permanent tooth enamel is covered with Nasmyth membrane that abrades when the tooth gets to the occlusal plane. A precipitate of salivary proteins and inorganic elements from buccal means is produced over this membrane forming a clear film with no bacteria that forms again a few hours after cleaning the tooth surface mechanically.

Dental arches are placed in a virtual box –the mouth–, covered by highly vascularized mucus that is permanently humid and with a temperature of about 36.6°, which provides many ecological niches for its flora.

Newborn's oral cavity is considered sterile until it inhales for the first time; from that moment, mouth flora increases rapidly within the first 8 hours.

Among the pioneering species, we found *streptococo salivarius* that specially colonizes the tongue and circulates through saliva. After some weeks of birth, buccal flora composition stabilizes and remains relatively constant.

The first primary teeth eruption creates the opportunity for the microorganisms adapted to live over hard surfaces establishing and growing, building up a dental plaque or bacterial plaque.

The oral mucus, the soft surface of dental crowns, and saliva through its washing function and the chemical action of its inhibitors are some of the factors that contribute to the oral cavity health.

Bacterial plaque forms by the selective adsorption of salivary glycoproteins onto tooth enamel surfaces. This layer is called acquired pellicle or acquired cuticle and forms again onto the dental tissue two hours after being completely removed. Its function is to avoid enamel surface desiccation and to diminish friction between tooth surfaces.

After some time, this cuticle changes by the apposition of gingival desquamated cells, polymorphonuclear leukocytes, bacteria present in the mouth or those entering through air or food.

The dental plaque or bacterial plaque has a well-defined structure formed by bacterial cells ordered in groups or columns of microcolonies, and epithelial and inflammatory cells between

which saliva, gingival fluid and food liquids pass through. Intercellular spaces fill with polysaccharides and substances synthesized by bacteria. This plaque is commonly whitish or yellowish, and forms close to dentogingival limits, within interdental spaces and on tooth surface irregularities.

If dental microbial plaque is not removed, it can mineralize to become calculus. According to some researchers, children do not tend to form calculus.

Dental plaque flora presents qualitative and quantitative differences between individuals, even between the different ecological niches of the same individual. The nature of this microbiota will determine the occurrence possibility of two of the most frequent dental diseases (dental caries and periodontal disease).

Dental plaque development on tooth surface follows a common pattern, with small differences between teeth and individuals.

On an early stage, there is a proliferation of cocci among which *Streptococcus sanguis* is the main colonizer. Later there appears *Actinomyces viscosus* together with other microorganisms among which streptococcus prevails.

This dental plaque has an aerobic metabolism and facultative aerobic bacteria.

The most aerobic bacteria are replaced by anaerobe and facultative anaerobe bacteria. Some authors underline the absence of *Porphyromonas* and *Prevotella* in the dental plaque of children under 12.

As the dental plaque gets older, the deepest parts will lack of nutrients and oxygen, gradually diminishing the number of living organism.

The dental plaque that remains adhered to the tooth surface for long periods can be invaded by chromatogen bacteria that alter its stain.

During primary and mixed dentition, it is rare to see dental plaques with black stains. The children with those black stains usually have low caries incidence.

This black-stained plaque is called **BLACK STAIN**, which consists in 1-mm line along primary and permanent teeth gingival contour during mixed dentition period.

The group of pigmented bacteria (*Prevotella* – *Porphyromonas*) is part of the normal flora in the oropharynx, nose and genitor-urinary and gastrointestinal systems, and are the second most significant anaerobic bacteria found in adult human infections.

However, those patients with exogenous **BLACK STAIN** do not show signs of mouth diseases such as dental caries or periodontopathy.

The **BLACK STAIN** appears early on the tooth enamel, at the age of 2 or 3, sometimes like dark spots close to the cervical edge of the tooth crown or like a continuous line. Some other times, it covers a bigger tooth area on the palatal or lingual surface of incisive teeth. Dentist consultation is usually related to aesthetic issues, especially after children entering school.

Extrinsic **BLACK STAINS** usually appear as a black line on tooth vestibular, lingual and palatal surfaces and gingival margin, or, in a more generalized way, as vague stains on the clinical crown.

PURPOSES

General

- To identify anaerobic pigment-forming bacteria in the buccal flora of children with deciduous and mixed dentition with *black stain*.
- To correlate its occurrence with dental caries incidence.

Specific

- To identify the anaerobic pigment-forming bacteria present in *black stain, dental plaque* and *saliva*.
- To measure saliva pH.

MATERIALS AND METHODS

A cross-sectional study was practiced to establish the correlation between the presence of pigment-forming bacteria in the mouth and *black stain*, and to evaluate its influence on caries index.

Accessible population: A total of 433 children, 195 girls and 238 boys, aged between 3 and 10 years old were examined at the *Servicio de Diagnóstico de la Facultad de Odontología de Rosario*, when they attended for the buccal health certification or for dental treatment referral.

The selected patients formed the accessible population and the number of tooth surfaces with *black stain* of every sampling unit became an observation unit.

That examined population was divided in 2 analysis groups:

- **GROUP No. 1:** Patients who had extrinsic *black stain* at the time of dental inspection. Those 18 children formed the study group.
- **GROUP No. 2:** Eighteen patients selected from the attendees who had no stained teeth.

Both groups had a medical records and a buccal-health card filled in.

A graphic record for *black stain* was kept for every child of Group No. 1, where it was registered vestibular and palatal surfaces for upper teeth, and vestibular and lingual surfaces for lower teeth.

Every tooth surface was illustrated segmented into three sectors - gingival, medium and incisal (or occlusal) - in order to record the pigmentation location and extension. The bacteriological exam was practiced at the Bacteriological Area of the Biochemistry and Chemistry School, Rosario National University.

Saliva, *dental plaque* and *black stain* were sampled from the children of Group N° 1; *saliva* and *dental plaque* were sampled from the children of Group N° 2.

All the samples were cultured in Brain Heart Infusion Blood Agar (BHIBA) supplemented with K-vitamin (10 µg/ml) and hemine (10 µg/ml), and another plaque was supplemented with ampicillin (50 µg/ml) to select the flora.

Saliva pH was measured with a Macherey-Nagel indicator strip 6.4-8.0, calibrated every 2 tenth. The samples were processed using the Gen bag Biomerieux anaerobic system for 48 hours, at 37° C.

RESULTS

Number of subjects examined by gender with or without *black stain*

Children examined	Gender		TOTAL	%
	F	M		
With B. S.	188	227	415	95.8
Without B. S.	7	11	18	4.2
Total	195	238	433	100

B. S: black stain

There are approximately 4 out of 100 registered patients.

The analysis of the 36 children's medical records allowed us to establish significant differences between patients from Group No. 1 and those from Group No. 2

Every child with *black stain* had problems in the respiratory tract:

- 1) 18 children had respiratory tract obstruction due to mucus secretion;
- 2) 15 children had bronchial spasms at least three times a year and were administered corticoid medication;
- 3) 11 children experienced angina and/or ear infection processes between 2 and 3 times a year and had to be treated with antibiotics.

The dental exam revealed some similarities between the two groups.

- 1) Caries index (DMF-T) =0;
- 2) Healthy gingiva without any kind of inflammation;
- 3) Proper oral hygiene.

But in Group N^o 1, apart from dental surfaces with *black stain*, the following was observed:

- 18 children with mouth breathing (the habit of breathing through the mouth)
- 13 of them did it permanently and 5 only when sleeping; 18 children had oral habits,
- 8 out of which sucked their thumb, 7 bit their nails (onychophagia) and
- 3 proved to have both habits; all the patients from the Study group had severe halitosis which was referred to as persistent even after brushing or rinsing.

Incisive and canine teeth pigmentation areas showed the greatest variation as regards the location of the stain, both in the upper teeth and the lower teeth.

In every case, the stain was close to the tooth gingival edge or neck, without touching the gums. On the tooth vestibular surfaces, it was observed at 1 mm from the gingivodental junction, while on the lingual and palatal surfaces it was found closer to the gingivodental junction.

In the Bacteriological Exam, a greater frequency of *Prevotella melaninogenicus* was observed in *black stain*, while on *dental plaque* and *saliva* the *Porphyromonas gingivalis* prevailed.

Porphyromonas asaccharolytic was only found in three patients' saliva, *Bifidobacterium* spp. was found in one child, and *Fusobacterium varium* in another.

For the percentage of stained surfaces:

On average, the percentage of stained surface per patient equals 16.93%, while approximately 75% of the patients proved to have at least 20% of stained surface.

We could say there is a significant difference in the stained surface percentage according to the types of habits, if mouth breathing is nocturnal and permanent. The percentage of stained surface for those who have permanent mouth breathing is significantly greater than for those who have this habit at night.

Patients' participation with thumbsucking is significantly higher (61.1%). This habit is correlated with 45% of stained surface, which represents over 20% of the cases under study.

It should be noticed that 72% of the patients had bacteria on *black stain* and *saliva* and 78% on *dental plaque*.

P melaninogenic bacteria is the one that most frequently appears on *black stain*, and also has a significant occurrence on *dental plaque* and *saliva*. However, on *dental plaque* and *saliva*, the most frequent is *P gingivalis*.

CONCLUSIONS

Out of a total of 433 children being examined, aged between 3 and 10, only 18 (4.16%) had extrinsic dental *black stain*, thus revealing a low prevalence of this type of condition.

Although the population under study was small, it allowed us to agree with what different researchers established -that the *black stain* wouldn't be a very prevailing condition.

In this study, children with black stain had DMF-T=0, indicating a direct relationship between *black stain* and a zero caries index. The pigmentation extension over the stained tooth surfaces was studied to assess the percentage of tooth affected surfaces, and we found a great variability.

The greatest number of tooth surfaces (65%) showed substantial pigmentation on a line 0.5 to 1 mm thick around the gingival edge. Therefore, it should be considered that due to the cross-sectional nature of this study, the recording of the condition was performed at the time of inspection, and some of these children stated they had received dental care with partial removal of the *stain*.

The percentage of stained surface per patient averaged 18.04% and half the patients showed a pigmentation percentage of at least 14.58%, there being no significant differences between the examined dentitions, which would suggest that *black stain* affects both deciduous and permanent teeth.

As all patients with *black stain* were in the habit of mouth breathing, the stained surface percentage was related to such habit by means of the Statistical Hypothesis Testing. It was then concluded that at least 95% of permanent infant mouth breathers showed a greater stain extension than those who only did it at night.

Since some of these children showed that they were in the habit of biting their nails (onychophagia) or of sucking up their thumb or other fingers (thumbsucking/fingersucking), both variables were separately related to the stained surface percentage. The patients who suffered or did not suffer from onychophagia did not show any statistically significant differences for the covered surface due to black pigmentation.

Sixty-one percent of the children in the study group had the habit of thumbsucking/fingersucking, and the tooth stained surface in these patients represented over 20% of the cases under study.

A high percentage of the studied population were found to be mouth breathers, both permanent and nocturnal, many of whom were also in the habit of fingersucking/thumbsucking. When related to pigmented tooth surfaces, both variables showed to be directly related.

The children in the Group No. 1 had problems in the respiratory tract; all of them presented obstruction due to retropharyngeal mucus; over 80% of them suffered from bronchial spasms and received corticosteroid therapy more than three times a year. Some of these children had infectious processes in their internal or middle ear, and anginas.

When these pathologies were related to the presence of anaerobic pigment-forming bacteria (whether genera *Prevotella* or *Porphyromonas*) in *black stain*, *dental plaque* and *saliva* in the study-group children, which, according to most authors, are not common in children's buccal cavity, it could be considered that there existed predisposing factors for the appearance of these microorganisms in the mouth and the subsequent tooth surface pigmentation.

It was observed that the deciduous second molars were the most affected by *black stain*, despite they only showed a condition classified as Grade 1.

The proximity of the rhinopharyngeal region with infected mucus could be related to these molar surfaces with plaque colonized by chromogenic bacteria.

The secondly affected areas were the upper central incisors, since they are not protected by saliva due to mouth breathing, which could have favored bacteria adhesion and colonization.

The presence of pigment-forming anaerobes could be related to these bacteria capacity to find their essential metabolites in the supporting flora, which would consume the oxygen present in the plaque, losing its cariogenic potential.

They act in a host with pathologies which compromise the respiratory and buccal tract, which would allow the confirmation of the relation between *black stain* and the low rate of dental caries.

FINAL CONCLUSIONS

The extrinsic tooth *black stain* constitutes a disease which is not so prevalent and which affects the deciduous and mixed dentition, generally disappearing once the permanent dentition is established. It is produced by the pigment elaborated by chromogenic anaerobic bacteria from the genera *Prevotella* and *Porphyromonas*.

Children with *black stain* show a low rate of caries and according to the present study, it is associated with respiratory tract pathologies: bronchial spasms, chronic retropharyngeal mucus, ear infection and oral habits such as fingersucking/thumbsucking and mouth breathing.

The findings of the present study would allow us to assert the need to remove the stained tooth surfaces with tools which do not damage the enamel structure in order to avoid the adhesion of new bacteria and to establish an interdisciplinary treatment involving a dentist, a pediatrician, a speech therapist, an otolaryngologist and a biochemist, forming a health team which could control the pathology and re-establish the physiological balance of the child in an integral way.