ALTERATIONS OF THE ORAL ECOSYSTEM IN CHILDREN WITH CELIAC DISEASE

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ABSTRACT

The aim of this work is to evaluate the alterations of the oral ecosystem in symptomatic children with celiac disease (CD), to establish a particular pattern of oral markers that can be used as presumptive diagnosis of CD. Material & Methods: A sample of n=52 children with CD diagnosis according to the modified criteria of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN), 1990, was studied. A dental clinical evaluation of soft and hard tissues was performed. Saliva samples were obtained; in which buffer capacity, total proteins, calcium and phosphate were measured and SDS PAGE 12% electrophoretic profiles were performed. In addition, oral mucosa smears were collected by brushing. Results: Low frequency of enamel structural alterations was found, particularly in the permanent teeth of children with CD. These alterations had characteristics of chronological coherence (31.7%), bilateralism (26.8%) and symmetry (29.23%). The celiac smears in the celiac group (20%) showed significant presence of polymorphic nuclei and free nuclei. The celiac group had significant differences in buffer capacity, IgA levels, minute volume, calcium and Ca/P ratio ($p<0.05$). The protein profiles of CD children showed the absence of bands of low, medium and high molecular weight. Conclusion: Our results enable us to develop an alteration pattern corresponding to the oral ecosystem of CD children. In the CD patients, the most relevant variables were tooth enamel alterations, oral mucosa morphology, and modifications of salivary parameters, which would enable the dentist to refer these patients to specialist physician.

Key words: celiac disease, saliva, oral ecosystem, presumptive diagnosis

ALTERACIONES DEL ECOSISTEMA BUCAL EN NIÑOS CELÍACOS

RESUMEN

El objetivo de este trabajo es evaluar las alteraciones del ecosistema bucal en niños sintomáticos con Enfermedad Celíaca (EC), para establecer un patrón de marcadores orales característico que pueda ser utilizado como diagnóstico orientativo de EC. Material y Métodos: Se estudió una muestra de n=52 niños con EC según criterios de European Society of Pediatric Gastroenterology, And Nutrition (ESPGAN) modificados 1990. Se realizó la evaluación clínica odontológica de los tejidos blandos y duros. Se obtuvieron muestras de saliva, en las cuales se midieron capacidad buffer, proteínas totales, calcio, fosfato y se realizaron los perfiles electroforéticos mediante SDS PAGE 12%. Además se recolectaron citologías exfoliativas por cepillado de mucosa oral. Resultados: se observó una baja frecuencia de alteraciones estructurales en el esmalte, particularmente en la dentición permanente en niños con Enfermedad Celíaca. Estas alteraciones se manifestaron con coherencia cronológica (31,7%), bilateralidad (26,8%) y simetría (29,23%). La citología exfoliativa en el grupo celíaco (20%) mostró la presencia significativa de polimorfonucleares y núcleos libres. En este mismo grupo se observaron diferencias significativas en la capacidad amortiguadora, niveles de IgAs, volumen minuto, calcio y relación Ca/P ($p<0,05$). Y a nivel de los perfiles de proteínas se observó la desaparición de bandas de bajo, medio y alto peso molecular en los niños celíacos. Conclusión: nuestros resultados nos permiten elaborar un patrón de alteraciones a nivel del ecosistema bucal de los niños con EC. Del conjunto de variables con mayor relevancia en los pacientes con EC fueron las relacionadas a características de las alteraciones del esmalte dentario, morfología de la mucosa oral y modificaciones de algunos parámetros salivales que permitiría orientar al profesional odontólogo a derivar a pacientes con este patrón al profesional médico competente.

Palabras clave: enfermedad celiaca, saliva, ecosistema bucal, diagnóstico presuntivo

INTRODUCTION

Systemic pathologies such as celiac disease (CD) can alter the oral ecosystem[1]. CD is a permanent, multifactorial disorder associated to genetic factors such as the autoantigens HLA-DQ2 or HLA-DQ8 of the T-cells and environmental factors such as the gluten from some cereals[2,3]. The pathology is characterized by subtotal or total villous atrophy of the small intestine, which interferes with nutrient uptake from food[1]. The main symptoms in infants

and young children are diarrhea and abdominal swelling, among others, which consequently delay growth. In addition, there may be extra-intestinal symptoms such as short stature, anemia, weight loss, joint pain, irritability, fatigue, mouth ulcers and structural alterations of teeth[1,3].

Current epidemiological data show that CD is common in the world, affecting not only European countries but also populations with European ancestry such as developing countries in southern Asia, South Africa and South America, in which prevalence is similar to that in Europe[2]. World prevalence of CD is about 1:300, but currently it has increased because of the recognition of the asymptomatic form, which is a health concern[4,5]. Prevalence in Argentina has been recorded in a single monitoring study conducted at Universidad de La Plata, and was established as 1:160 individuals[6]. Genetic predisposition to CD has been studied in family groups, and it was found that their risk of CD is 20 to 30 times greater than it is in the rest of the general population[2]. Early identification of CD enables treatment with gluten-free diet to be started, reducing the risk, albeit low, of developing gastrointestinal lymphomas or carcinomas[1-5].

Certain diagnosis of CD is done by means of an intestinal biopsy following the ESPGAN criteria, since other tests, such as genetic and/or immunological tests, are not conclusive for diagnosis[5]. Today, the establishment of non-invasive monitoring methods is being attempted to support the prescription of jejunal biopsy[7], a technique which is particularly hard on children. Non-invasive methods could include odontological variables, particularly pertaining to oral mucosa, because they are easily accessible, easy to evaluate and low-cost. CD patients are known to have certain odontological clinical features that can be used as markers for predicting the disease[8]. Regarding oral alterations, some authors have reported that CD patients suffer from recurrent aphthous stomatitis, mouth ulcers and/or autoimmune diseases with oral manifestations, even celiac patients on gluten-free diet[1,7]. Another study found no statistically significant differences of positive histories of aphthous ulceration between patients diagnosed with CD and a control group[9].

Regarding dental tissues, studies by Prati et al.[10] report a statistically significant delay in temporary tooth eruption in children with CD compared to a control group. The same study[10] showed that patients with CD are equally susceptible to having caries as the general population, while other studies report that when CD is associated to other pathologies, caries incidence is higher[11]. Furthermore, other authors[10-12] have described greater prevalence of structural defects of tooth enamel in patients with CD. Another major component in the oral ecosystem is saliva, which maintains homeostasis by means of proteins such as secretory immunoglobulin A (IgAs), immunological factors, non-immunoglobulin factors (lactoferrin, lisozyme and the peroxidase system), T-cells with specific regulatory properties and the cell traffic system, among others[13]. Studies of whole saliva[13] showed that CD patients have a significant increase in total protein concentration of salivary IgAs and IgG compared to healthy controls. Authors such as Al-Bayaty et al.[14] reported presence of IgA anti-gliadin (IgA AGA) antibodies in saliva, suggesting that this could be applied for the prescription of biopsies in children and adults.

The aim of this study is to assess alterations in the oral ecosystem in symptomatic children with CD to establish a characteristic pattern of oral markers that could be used as a suggestive diagnosis for CD.

MATERIALS AND METHODS

We studied a sample of n=52 children with CD according to the criteria of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) modified in 1990[15], diagnosed by the gastroenterology specialist physician by means of intestinal biopsy as grade III or IV, considering the small intestine as normal when the villous height: crypt depth ratio is approximately 4:1 to 5:1[16], and n=23 healthy children as a control. Both study groups included children of both sexes aged 4 to 12 years who attended the Gastroenterology Service at "Santísima Trinidad" Children's Hospital in Córdoba city, Argentina, and the Department of Pediatric Dentistry at the School of Dentistry, Universidad Nacional de Córdoba, Argentina, during 2004-2005. All medical, odontological and laboratory clinical data were recorded in a single clinical history. Healthy controls were children not affected by gastrointestinal pathology or other diseases that may be related to CD, and whose routine serological examinations at the hospital showed negative IgG antigliadin (Ig AGA) and antiendomysial (IgG EMA). Individuals who at the time of the study were taking any type of medication acting on the immunological system were excluded from the study, as were any who had another immunological disease and/or serious periodontal disease. This study was approved

by The Research and Ethics Committee of the Ministry of Health of Córdoba Province, and followed the guidelines of the Declaration of Nuremberg, Helsinki, Tokyo of the World Medical Association.

Clinical-odontological examination: conducted by means of routine instrumental exploration, with artificial lighting, after cleaning the teeth with a toothbrush and drying them. The following were observed: a) *Characteristics of soft tissues;* inspection of lip, cheek, soft-hard palate, tongue, floor of the mouth, gums, presence of **aphthae** according to the Grispan criteria[17]; b) *Characteristics of hard tissues*; **oral hygiene** according to the simplified Greene and Vermillion index[18], **degree of gingival inflammation** according to the gingival health index proposed by Löe and Silness[19], **eruption period** of temporary and/or permanent teeth according to Logan and Kronfeld's table, considering as normal any variations of ± 6 months from the usual eruption date for each tooth[20], dmft and DMFT according to Klein et al.[21], **structural enamel alterations**, considering both simple dyschromia and anomalies in the shape of the tooth and symmetrical, bilateral and chronological distribution[22].

Oral mucosa smears: oral mucosa smears were taken by brushing (Cytobrush) from the non-keratinized zones of cheek and/or lips of celiac and healthy children. Smears were fixed in 96ºC alcohol for 1 - 2 hours, and then stained with hematoxylin/eosin. They were studied under an Olympus BX50 optical microscope, photographed using a SONY video camera and processed using Image Pro-plus software. The histopathological characteristics of the samples were described according to the classification of the World Health Organization (WHO)[23].

Sialochemical studies: samples were taken of saliva stimulated with sugar-free mint-flavored Beldent chewing gum with xylitol (Stani®), for 5 minutes, two hours after food intake, by direct salivation into a graduated polyethylene tube and kept in ice until processed. *Salivary flow rate* (mL/minute) was calculated and pH immediately determined using the potentiometric method (Orion pH-meter). *Buffering capacity* was determined as the difference between pH values before and after adding 1ml of 5 mM-HCl to 1 ml of saliva, and the smaller the change in pH, the greater was saliva buffering capacity[24]. Levels of *total protein*[25], *calcium*[26] and *phosphate*[27] *fluoride* were measured using an electrode selective to the fluoride ion, following Duckworth's technique[28] and *secretory IgA*[29]. The *protein profile* was obtained by electrophoresis with 12.5% SDS PAGE according to Laemmli's method[30] using 1:1 aliquots of saliva supernatant centrifuged in buffer. 50µg of the sample were placed on each path and the gels were stained with 0.1% Coomasie Blue R-250.

Microbiological study on saliva: levels of *Streptococcus mutans* were determined using MSB Agar culture medium (Agar *Mitis Salivarius*, Difco Laboratories, USA, +0.2 U/ml de Bacitracine + 15 g % sucrose) + 0.1 ml potassium tellurite/100 ml culture medium. *Streptococcus mutans* ATCC 35668 and *Streptococcus mitis* ATCC 6249 were used as a control and bacteria were counted after 72 hours. The counts were reported in CFU/ ml saliva (colony-forming units per ml of saliva): *High Level*; $> 10^6$ CFU/mL, *Medium Level*; 10^3 to 10^6 CFU/mL, *Low Level*; $<10^3$ CFU/mL. The activity of *Lactobacillus spp.* was determined in Rogosa Agar medium (Biokar Diagnostics, France). Plates were inoculated with pure *Lactobacillus spp* strains as a control. Bacteria were counted after 72 hours. The counts were reported in CFU/ml saliva (colony-forming units per ml of saliva): *High Activity*; $>10^4$ CFU/mL, *Medium Activity*; 10^3 to 10^4 CFU/mL; *Low Activity*; $<10^3$ CFU/mL[31].

Statistical analysis: all data were described by centralization measures (mean/median) and measures of variance (standard error). The data obtained from qualitative variables were processed statistically using the Wilcoxon non-parametric test, while the Student T-test was used for data from quantitative variables. In all cases, the critical level of significance was set at $p \leq 0.05$ for establishing significant differences[32].

RESULTS

Clinical odontological examination: 100% of patients with CD and healthy controls had no stomatological lesions in oral mucosa. However, 63.15% of the celiac patients reported they had had them at some time in their lives. In general, the eruption of temporary and permanent teeth, and indicators of caries experience, oral hygiene and gingival health of the population under study showed no significant variations in children with CD compared to healthy children (Table 1). Children with CD had a low frequency of structural enamel alterations, which were present particu-

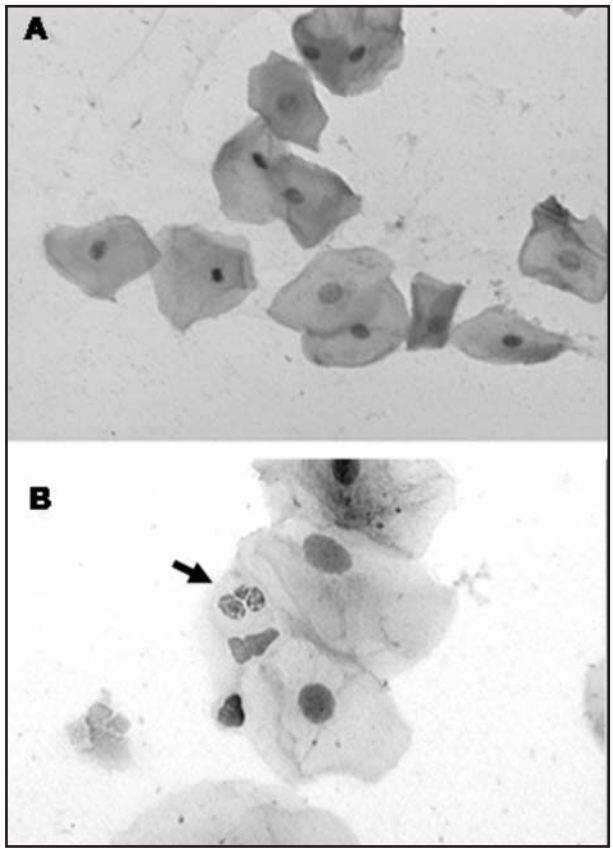

Fig. 1: Oral mucosa smears under optical microscope, stained with H/E. A) Control patient (x200); B) Celiac patient. Arrow: leukocyte (x400).

larly in permanent teeth. However, their permanent teeth showed chronological coherence (31.7%), bilateralism (26.8%) and symmetry (29.23%).

Oral mucosa smears: The mucosa of the cheek and/or lip showed significant morphological differences ($p<0.05$) compared to the control group. The celiac group (20%) showed polymorphic nuclei and free nuclei (PMN) counted in 50 mm^2 fields at 400X magnification (Fig. 1).

Sialochemical studies: saliva measurements such as buffering capacity, IgAs levels, minute volume, calcium and Ca/P ratio reveled statistically significant differences ($p<0.05$) in buffering capacity (Table 2). Protein profiles showed disappearance of bands of low, medium and high molecular weight in celiac children (Fig. 2).

Microbiological study on saliva: For all groups, the *Streptococcus mutans* count showed that the highest percentage was found at the medium level of microorganisms, while high levels were not found. *Lactobacillus* spp activity was also greatest at the middle level for all groups.

Table 1: Evaluation of hard and soft tissues expressed as a percentage (%), calculated as number of children at a given level / total number of celiac or healthy children. (*) Categories >0 indicates caries experience at some time. (**) Indicates statistically significant differences, $p<0.05$ among all groups compared.

Variable studied	Levels	Celiacs (%) (n=52)	Control (%) (n=23)
Eruption of temporary teeth	Normal	69.0	69.5
	Late	8.3(**)	13.0
	Early	0(**)	13.5
Eruption of permanent teeth	Normal	56.9	60.0
	Late	23.7	25.0
	Early	12.0	15.0
Caries experience*	dmft > 0	67.0	65.5
	DMFT >0	42.8	42.9
Oral hygiene	Good	65.5	62.0
	Fair	34.5	32.0
Gingival Health	Slight	61.9	56.0
	Moderate	38.0	44.0

DISCUSSION

The increase in asymptomatic forms of CD is currently encouraging the study of variables that could be used to monitor the disease, particularly in groups at risk, because late diagnosis has a negative impact on the life quality of patients. Within this context, the study of components of the oral ecosystem is of great interest for predicting CD.

Regarding tooth tissues, our results show that both primary and permanent tooth eruption periods were normal. In contrast, authors such as Prati et al.[10] found a statistically significant delay in temporary tooth eruption in patients with CD, with no distinction between patients exposed and not exposed to gluten. These authors consider that the general or local causes responsible for late eruption may be poor uptake; therefore there might be a relationship between CD and mean age of temporary tooth eruption in these patients compared to healthy children. In agreement with our observations, Prati et al.[10] reported that the normal mean age for permanent tooth eruption in these children was not significantly higher than in healthy children. In addition, our research found a low percentage of structural enamel alteration in permanent teeth. Regarding this factor, literature reports contradictory results; studies by Aine et al.[22] of celiac adults

Table 2: Salivary components in celiac patients and healthy controls (average values ± SE).
(*) Indicates statistically significant differences between salivary measures in celiac children and the control group (p<0.05).

Salivary components	Celiac (n=52)	Healthy (n=23)
Total volume	4.083±0.232	3.786±0.384
Minute volume (ml/min)	0.825±0.081	0.813±0.089 *
pH	7.32±0.052	7.37±0.056
Buffering capacity	0.980±0.106	0.701±0.101 *
Proteins (g/L)	1.353±0.129	2.604±0.278
Secretory IgA (mg %)	10.28 ±8.01	14.90 ±5.22 *
Fluoride (mg)	0.009±0.002	0.011±0.002
Phosphate (mg %)	7.216±0.563	6.950±0.533
Calcium (mg %)	3.468±0.408	4.374±0.603 *
Ca/P ratio	0.606±0.085	0.728±0.708 *

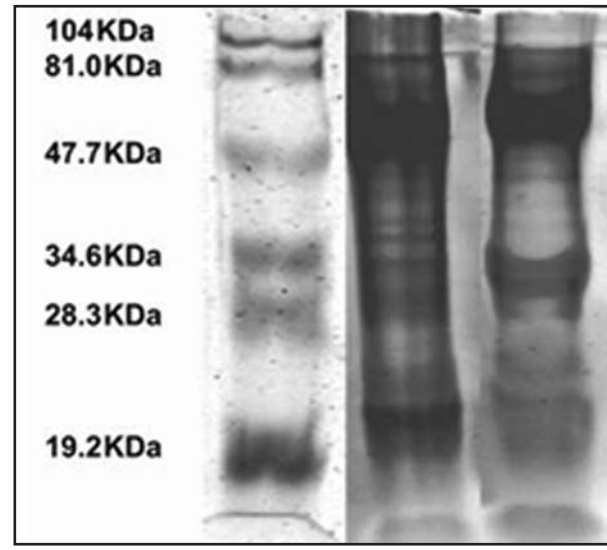

Fig. 2: Profile of total salivary proteins from the population under study by electrophoresis with 12% SDS. From left to right PM: Pattern of molecular weight, control, celiac patient.

report a high percentage of these lesions, whereas another study[33] of children and teenagers reports no significant difference between control and celiac groups. Nevertheless, recent studies[12] mention structural enamel defects as possible odontological markers for CD. Perhaps the low number of structural lesions found in our study is due to the fact that in most of the children, the symptoms began to appear before they were one year old, enabling them to start early on a gluten-free diet, and favoring amelogenesis.

Children with CD showed no clinical evidence of aphthae, although a high percentage (63%) of parents and/or persons responsible for the patients reported that they had had them prior to clinical inspection. This finding matches the reports by Bertoldi et al.[34], Petrecca et al.[35] and Lähteenoja et al.[36], who concluded that the presence of aphthous lesions are characteristic of CD. Similarly, research by Sedghizadeh et al.[9] showed the presence of aphthous ulceration in patients with CD.

In general, systemic pathologies can affect the flow and/or composition of salivary secretion[37]. Regarding salivary proteins, no significant difference ($p>0.05$) was found between saliva concentrations in children with CD and healthy children; nevertheless, the electrophoretic profile showed a reduction of the bands with molecular weights between 47 and 19 KDa. In agreement with our study, other authors[13] reported an increase in minute volume in celiac children compared to healthy controls, while total salivary volume did not vary significantly. Regarding inorganic salivary components, the drop found in values of calcium, Ca/P ratio and buffering capacity in celiac children compared to healthy controls might be related to the alteration of bone metabolism typical of this disease, which has been widely documented[5]. Furthermore, our previous studies show that the variables proteins and Ca/P molar ratio are significantly associated to the celiac condition[5].

Within the soft tissue factors studied, the findings related to oral mucosa morphology were the most relevant, because patients with CD had presence of PMN, whether they reported being on a gluten-free diet or not. In general, the literature reports presence of PMN in smears from subjects with infectious processes and/or other systemic diseases involving the immune system[38]. However, the conditions under which the morphology of oral mucosa should be considered as a marker for presumptive CD need to be properly established, so further transversal and longitudinal studies are needed to clarify its alterations, also considering patients with different symptoms and whether their diets are gluten-free or not.

Celiac disease has multifactorial etiology; therefore it would be of interest to conduct multivariate studies to analyze a set of variables. Notwithstanding, studies of odontological factors related to celiac disease have been conducted considering them separately[14, 22, 33, 36].

To sum up, our results allow us to develop a pattern of alterations of the oral ecosystem in children with CD. The most relevant set of variables in patients with CD was related to the characteristics of alterations in den-

tal enamel, morphology of oral mucosa and alterations of some salivary measures that allow the dentist to refer patients with this pattern to a competent physician. In addition, children diagnosed with CD have salivary parameters especially associated to the descent in the capacity for buffering, mineralizing and defense; therefore they should be enrolled in a follow-up protocol for patients at risk regarding oral health.

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