Analysis of oral health and salivary biochemical parameters of women with anorexia and bulimia nervosa

Análise da saúde bucal e parâmetros bioquímicos salivares de mulheres com anorexia e bulimia nervosa

Análisis de parámetros bioquímicos de salud bucal y salival de mujeres con anorexia y bulimia nerviosa

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Abstract
Objective: The aim of this study was to analyze the association between caries, dental erosion and salivary biochemical profile of women with anorexia and bulimia, comparing them with a control group. Methods: The participants were women from the Eating Disorders Program of the Mental Health Clinic. We evaluated caries and dental erosion and collected stimulated saliva samples for the analyses of salivary flow, phosphor, calcium, acid and alkaline phosphatase, amylase, FRAP and uric acid. Data were expressed as means and standard deviations and were analysed using Mann-Whitney test with $p < 0.05$ regarded as significant. Results: 13 women with anorexia and bulimia participated in the study, with an mean age of 28.9 years, and 15 comprised the control group. The DMFT index was 15.5 for the case group and 4.0 for the control group. Erosion was found mostly on the palatal/lingual surface and on incisor teeth of case group. Most biochemical analyzes showed a significant difference between the two groups. Conclusion: We concluded that, in patients with anorexia and bulimia, due to a decreased salivary flow and self-induced vomiting habits, there is an increase in the caries and dental erosion index, as well as salivary changes.
Keywords: Anorexia; Bulimia; Eating disorders; Oral manifestations; Saliva.

Resumo
Objetivo: O objetivo deste estudo foi analisar a associação entre cárie, erosão dentária e o perfil bioquímico salivar de mulheres com anorexia e bulimia, comparando-as com um grupo controle. Métodos: As participantes eram mulheres do Programa de Transtornos Alimentares da Clínica de Saúde Mental. Avaliamos cárie e erosão dentária e coletamos amostras de saliva estimulada para análises de fluxo salivar, fósforo, cálcio, fosfatase ácida e alcalina, amilase, FRAP e ácido úrico. Os dados foram expressos em média e desvio padrão e analisados pelo teste de Mann-Whitney com p <0,05 considerado significativo. Resultados: Participaram do estudo 13 mulheres com anorexia e bulimia, com média de idade de 28,9 anos, e 15 constituíram o grupo controle. O índice CPOD foi de 15,5 para o grupo caso e 4,0 para o

grupo controle. A erosão foi encontrada principalmente na superfície palatina/lingual e nos dentes incisivos do grupo caso. A maioria das análises bioquímicas mostrou uma diferença significativa entre os dois grupos. Conclusão: Concluímos que, em pacientes com anorexia e bulimia, devido à diminuição do fluxo salivar e hábitos de vômitos autoinduzidos, ocorre aumento do índice de cárie e erosão dentária, bem como alterações salivares.
Palavras-chave: Anorexia; Bulimia; Distúrbios alimentares; Manifestações orais; Saliva.

Resumen
Objetivo: El objetivo de este estudio fue analizar la asociación entre caries, erosión dental y perfil bioquímico salival de mujeres con anorexia y bulimia, comparándolas con un grupo control. Métodos: Las participantes fueron mujeres del Programa de Trastornos de la Alimentación de la Clínica de Salud Mental. Evaluamos caries y erosión dental y recolectamos muestras de saliva estimulada para los análisis de flujo salival, fósforo, calcio, fosfatasa ácida y alcalina, amilasa, FRAP y ácido úrico. Los datos se expresaron como medias y desviaciones estándar y se analizaron mediante la prueba de Mann-Whitney con p <0,05 considerado significativo. Resultados: participaron en el estudio 13 mujeres con anorexia y bulimia, con una edad media de 28,9 años, y 15 constituyeron el grupo control. El índice CPOD fue de 15,5 para el grupo de casos y 4,0 para el grupo de control. La erosión se encontró principalmente en la superficie palatino / lingual y en los incisivos del grupo de casos. La mayoría de los análisis bioquímicos mostraron una diferencia significativa entre los dos grupos. Conclusión: Concluimos que, en pacientes con anorexia y bulimia, debido a la disminución del flujo salival y los hábitos de vómito autoinducido, existe un aumento del índice de caries y erosión dental, así como cambios salivales.
Palabras clave: Anorexia; Bulimia; Trastornos de la alimentación; Manifestaciones orales; Saliva.

1. Introduction

Eating disorders are behavioral syndromes characterized by changes in eating habits and detrimental behaviors to health. Their etiology is multifactorial and determined by biological, genetic, psychological, sociocultural and family components (Kessler et al., 2013).

They are marked by abundant or deficient food intake due to the individuals' view of a distorted body image on the cognitive or perception level. The individual stops eating in a healthy and balanced way and associates this attitude with aggressive methods for pursuing a decrease in body weight, which originates severe health problems. Among the main disorders are anorexia and bulimia, which are habits that become secret and ritualized. (APA 2014; Gomes & Silva 2010).

Anorexia nervosa is defined as a behavioral disorder characterized by deliberate, self-imposed starvation, followed by a constant pursuit of thinness and a morbid fear of gaining weight, which leads to serious levels of weight loss. The individual starts prioritizing his/her diet, weight and body shape (APA 2014). This disorder can be of the restricting or purging type. In the restricting type, there are no episodes of binge eating and no behaviors such as self-induced vomiting and use of medications, while the purging type presents episodes of binge eating and the use of laxatives and diuretics (Assumpção & Cabral 2002).

Bulimia nervosa is characterized by recurring episodes of binge eating (known as bulimic episodes), followed by inadequate compensatory behaviors such as self-induced vomiting, use of laxatives and diuretics, and excessive exercising, fasting and restrictive dieting, with the aim to achieve the desired thinness. Vomits, when too frequent, lead to a loss of the vomiting reflex, a relaxation of the lower esophageal sphincter and, in very advanced cases, may be spontaneously induced (APA 2014; Assumpção & Cabral 2002).

These behaviors lead to clinical complications of great interest for dental health due to causing several oral changes, such as dental caries, periodontal disease, dental erosion (also known as perimolysis), constant irritation of the oral mucosa and biochemical changes in the saliva (Panico et al., 2018).

Oral manifestations appear according to the frequency of the habits of each disorder, with emphasis on self-induced vomiting, hypercaloric diet, lack of hygiene after binge eating, anxiety, depression and the duration of the disorder (Barboza, Morais, Alves, Carneiro & Moura, 2011).

Knowledge about these pathologies must be of special interest among dental surgeons, as they play a relevant role in

the early diagnosis of eating disorders. With the help of intra and extra-oral exams, anamnesis and laboratory exams, the dentist can detect characteristic signs and symptoms of these conditions. Therefore, he/she can be the first health professional to discover the eating disorder (Navarro, Matoba Júnior, Tedeschi Filho & Queirós, 2011).

Within this context, this study aims to analyze caries, dental erosion and the salivary biochemical profile of women diagnosed with anorexia and bulimia of an Eating Disorders Program of a Mental Health Clinic, comparing them with the control group.

2. Methodology

Participants and study procedure:

The STROBE guidelines were followed in the preparation of this manuscript (Von et al., 2007).

This is a case control, descriptive research of analytical nature and with a quantitative approach. We studied women diagnosed with anorexia and bulimia (CID-10/F-50) in the Eating Disorders Program of the Mental Health Clinic of the Marília Medical School (FANEMA), São Paulo, Brazil, and a control group comprising women with the same characteristics, but without any type of eating disorder.

We excluded from the study women in menopause, undergoing hormone replacement therapy or using medications that change the levels of salivary flow, and those who did not agree to participate in the research. Data were collected from February to July 2018.

Ethical aspects:

The study was approved by the Research in Humans Ethics Committee, within the standards required by Resolution 466/12, CAAE process no. 80497417.1.0000.5420. All participants signed an Informed Consent Form.

Oral clinical exam:

The participants underwent a clinical exam in order to assess:

- Dental caries: Evaluated through the Dental Condition and Treatment Needs Index (DMFT). This exam checks the number of decayed, missing and filled teeth in the permanent dentition. The values of the index correspond to the following degrees of severity: very low (0.0 to 1.1), low (1.2 to 2.6), moderate (2.7 to 4.4), high (4.5 to 6.5) and very high (6.6 and higher). We adopted the codes and criteria of the Oral Health Surveys Basic Methods Manual (OMS 1999).

- Dental erosion: Determined by the Tooth Wear Index (TWI) proposed by Smith and Knight in 1984 (Smith and Knight 1984). Each tooth was assessed individually, including the vestibular, lingual and occlusal/incisal surfaces.

Exams were carried out by a single calibrated examiner (Kappa = 0.98) and an annotator, under natural light, using 1 (one) flat mouth mirror with grip and 1 (one) CPI (Community Periodontal Index) probe to aid the visual and tactile examination of the dental tissue (WHO 1999). We used all the personal protective equipment (PPE) recommended for each procedure.

Collection, processing and storage of saliva samples:

The material was collected approximately two hours after the last meal of the study participant during the morning period. The time of collection was chosen based on the fact that saliva samples could be affected by the presence of food and by the circadian cycle. All samples were collected in individual sessions under the same conditions.

The collection of stimulated saliva samples was carried out based on the following steps: The participant was

accommodated in a sitting position and told to chew a piece of plastic tape measuring 1.5 cm² (Parafilm M®, American National Can, Chicago, Illinois, United States) for one minute. The salivary content collected during this period was expectorated, and over the next five minutes the participant continued to chew the plastic tape and deposit the saliva inside the container with her head tilted down. The saliva samples were centrifuged at $5500 \times g$ for 10 minutes in a centrifuge refrigerated at 4º C to remove cell and food debris, squamous cells and insoluble contaminants. The saliva supernatant from each volunteer was divided into nine parts and frozen at -70° C for further analysis.

Biochemical analysis of the saliva:

The biochemical analyses were carried out with the saliva samples collected from each participant. The salivary flow was measured considering the value of 1.0 for the density of total saliva, while its ratio (mL/min) was calculated through the difference between the weight of the container before and after the collection, divided by the duration of the collection (Schutz, Utumi, Ignácio, Brancher & Fregoneze, 2017).

The salivary concentration rates of total protein were used to stabilize samples, following the Lowry method modified by Hartree (1972) and expressed in mg/l. The dosage of calcium and phosphor was done through colorimetry using Bioclin's® commercial kits (cresoftalein complexone method, Quibasa Química Básica Ltda., Belo Horizonte, Brazil). The modified colorimetry method was carried out for the biochemical analysis of the acid and alkaline phosphatase (Cruz, Oliveira, Sá & Corassin, 2017). The enzymatic activity of amylase was determined by the method described by Howe and Elmslie (1971) using Labtest's commercial kit. The total antioxidant capacity of the saliva was determined by the Ferric Reducing Antioxidant Power method (FRAP) described by Benzie and Strain (1996). The uric acid was evaluated using the Labtest kit, following the manufacturer's instructions.

Statistical analysis:

Data were expressed as mean ± standard deviation, and the variables were compared through the Mann-Whitney test was used to compare the groups with a significance level of 5% ($p<0.05$). The data were charted using Excel and the analysis was carried out through the software BioStat 5.3 (Ayres, Ayres Júnior, Ayres & Santos, 2007).

3. Results

The sample comprised 28 patients distributed into two groups: 13 women diagnosed with anorexia and bulimia nervosa, with an mean age of 28.9 years (sd=9.8), and 15 women with the same characteristics but without any type of eating disorder. Participants in the control group were selected from the companions of patients in the group with anorexia and bulimia nervosa following a consultation at the Eating Disorders Program of the Mental Health Clinic.

Regarding the clinical characteristics of patients, we examined 321 teeth in the case group and 420 in the control group. Tables 1 and 2 contain the distribution of severity and prevalence of erosive injuries according to the region and different types of teeth groups.

Table 1. Distribution of severity and prevalence of erosive injuries according to the region of the tooth.

DEGREE/ SEVERITY	CASE GROUP					
	Vestibular		Incisal/Occlusal		Palatal/Lingual	
	n	%	n	%	n	%
0	260	80.9	214	66.6	184	57.3
1	61	19.1	67	20.9	51	15.9
2	0	0	40	12.5	56	17.4
3	0	0	0	0	30	9.4
4	0	0	0	0	0	0
TOTAL	321	100	321	100	321	100
DEGREE/ SEVERITY	CONTROL GROUP					
	Vestibular		Incisal/Occlusal		Palatal/Lingual	
	n	%	n	%	n	%
0	400	95.2	392	93.3	392	93.3
1	15	3.6	28	6.7	28	6.7
2	5	1.2	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
TOTAL	420	100	420	100	420	100
p value	< 0.05		< 0.05		< 0.05	

Source: Authors.

Table 2. Distribution of severity and prevalence of erosive injuries according to teeth groups.

	CASE GROUP					
Teeth Groups	With erosion		Without erosion		Total	
	n	%	n	%	n	%
Incisives	88	97.8	2	2.2	90	100
Canines	37	77.1	11	22.9	48	100
Pre-Molars	13	14.8	75	85.2	88	100
Molars	2	2.1	93	97.9	95	100
	CONTROL GROUP					
Teeth Groups	With erosion		Without erosion		Total	
	n	%	n	%	n	%
Incisives	10	8.4	109	91.6	119	100
Canines	4	6.7	56	93.3	60	100
Pre-Molars	8	6.9	108	93.1	116	100
Molars	12	9.6	113	90.4	125	100
p value	< 0.05		< 0.05		< 0.05	

Source: Authors.

According to the Tooth Wear Index (TWI), we can see in Tables 1 and 2, no cases of maximum severity were detected in the patients. The most affected regions were the lingual surfaces and incisive teeth, showing significant differences between the groups.

Regarding the DMFT index in the same teeth analyzed previously, Table 3 presents each component separately, the mean and the p value, showing a significant difference between the two groups. In the group of women with anorexia and bulimia, the index was classified as "very high", while it was "moderate" in the control group.

**Table 3.** Percentage distribution of teeth according to the DMFT index.

Criteria	DMF-T Index				DMF-T Mean	Standard deviation
	Decayed	**Missing**	**Filled**	**Total**		
CASE GROUP	16	69	116	201	15.46	7.95
CONTROL GROUP	0	0	60	60	4.00	5.87
P value	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Source: Authors.

The salivary flow was very low in the group of women with eating disorders, whose mean was 1.17 mg/min, considered low when compared to the control group, whose mean was 1.71 mg/min (<0.05).

Regarding the salivary parameters, we carried out analyses with calcium and phosphor mg/dL (Graph 1), which showed a significant difference.
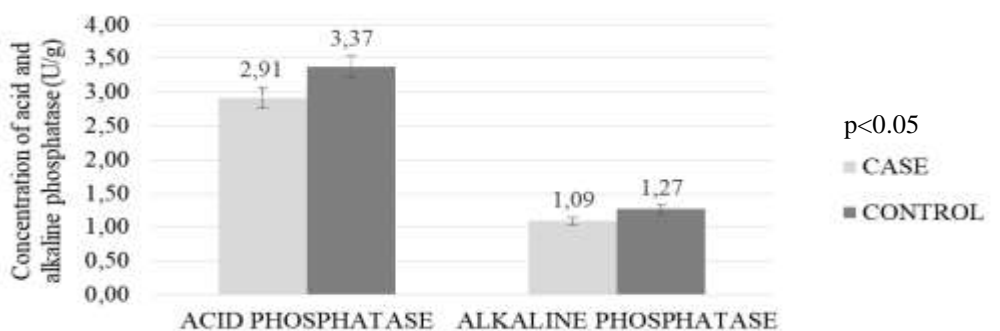
Graph 1. Percentage distribution of the concentration of phosphor and calcium in the saliva of patients with eating disorders and of the control group.
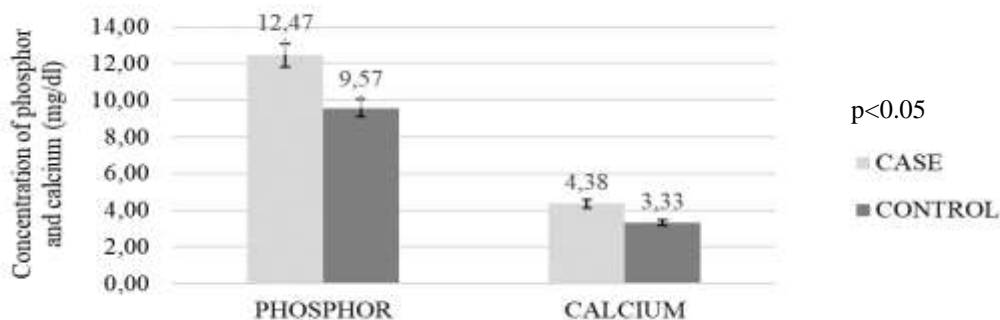
Source: Authors.

In the analyses of the acid (p=0.4642) and alkaline (p=0.6124) phosphatase, we verified no significant differences between the salivary parameters (Graph 2).

Graph 2. Percentage distribution of the concentration of acid and alkaline phosphatase in the saliva of patients with eating disorders and of the control group

Source: Authors.

Regarding the biochemical analyses of amylase, FRAP and uric acid, there was a significant difference in the two latter (Table 4)

Table 4. Percentage distribution of the concentration of amylase, FRAP and uric acid in the saliva of patients with eating disorders and of the control group.

Exams	Amylase		FRAP		Uric acid	
	Case	Control	Case	Control	Case	Control
Mean	654483.25	1033357.71	29.00	19.94	20.16	12.78
Standard deviation	83249.71	62515.29	12.98	6.64	10.93	4.93
p value	> 0.05		< 0.05		< 0.05	

Source: Authors.

4. Discussion

Their target population include especially female teenagers and young adults. The prevalence of anorexia is estimated between 0.3% and 0.9%, and of bulimia, between 1% and 2% of the general population. There are also important differences in the distribution among genders, with a men/women ratio that varies from 1:10 to 1:15 for anorexia and from 1:15 to 1:20 for bulimia. Researchers suggest, however, that the rates of prevalence for these disorders are underestimated, either due to methodological limitations or due to not including partial syndromes and risk behaviors for the development of eating disorders (Swanson, Crow, Grange, Swendsen & Merikangas, 2011).

This fact corroborates the findings in this research. The study was carried out only with women, because they were the vast majority of patients undergoing treatment in the mental health clinic. A research study by Carvalho, Val, Ribeiro and Santos (2016) also studied only women of a corresponding age range due to the prevalence.

The population studied has the frequent habit of self-inducing vomit, a fact that generates one of the main effects from the dental care point of view, which is dental erosion. It consists in an injury characterized by the dissolution of the mineralized tissues of the teeth caused by acids that do not involve bacteria. This condition is related to exposure to stomach acids resulting from regurgitations (Kunde, Mitinguel, Bellato & Moreira, 2017; Marshall 2018). Jarvinen, Rytomaa and Heinonen (1991) observed that the risk of erosion quadruples when regurgitation happens weekly, and it is up to eight times higher in patients with chronic vomiting. Scheutzel (1996) states that this clinical manifestation does not happen before the gastric acid starts coming into contact with the teeth on a regular basis, many times per week, during a period of 1 or 2 years. This research showed a significant difference between the groups studied, where patients who presented one of the types of eating disorders had a high erosion index, while the control group presented almost insignificant percentages, corroborating the findings of other researchers (Rytömaa, Järvinen, Kanerva & Heinonen, 1998; Lourenço, Azevedo, Brandão & Gomes, 2018), who associated this event and its severity with such disorders.

A study shows that erosion affects mainly the palatal and occlusal surfaces of the upper teeth, and that the lingual surfaces of the lower teeth are safe from the contact with the gastric acid due to being covered and protected by the tongue (Antunes, Amaral & Balbinot, 2007). In this study, we observed that the teeth and surfaces with most erosion were the incisives and canines in the palatal/lingual and incisal/occlusal regions of the patients with eating disorders. In addition, the areas with most wear due to mineral losses are more likely to develop dental caries, since bacteria can easily colonize the demineralized areas and penetrate the interior of the dentin (Neel et al., 2016).

In the case of eating disorders, the prevalence of dental caries is a controversial topic of multifactorial nature (DeBate, Tedesco & Kerschbaum, 2005). It is believed that cases of caries vary from one individual to another, and that there are several

factors influencing the cariogenic process, such as dental hygiene, the cariogenicity of the diet, malnutrition, fluoride exposure, genetic predisposition and the use of certain medications that influence salivary flow, especially antidepressants, which are common in the psychiatric treatment of these patients, and also appetite suppressants (Frydrych, Davies & McDermott, 2005). The author did not find significant differences regarding the index of dental caries between the populations (Brandt et al., 2017). On the other hand, the studie by Lourenço et al. (2018) found significantly high scores. This fact corroborates the findings of this study, where we found a high index of teeth affected by dental caries in the population who had one of the types of eating disorder. This difference appeared in the DMFT mean and in the components analyzed separately (decayed, missing and filled).

In this study, the analysis of salivary flow was significantly different between the groups, where women with anorexia and bulimia presented a lower quantity in mg/min when compared to the control group, corroborating the studies by Rytömaa et al. (1998) and Frydrych et al. (2005), who emphasized that the quality and composition of saliva influence susceptibility to oral diseases. On the other hand, Scheutzel and collaborators (1996) did not demonstrate this comparison in their study.

There are other analyses of salivary biochemical parameters that act as auxiliary tools in the diagnosis of clinical oral afflictions. In this study, we analyzed calcium, phosphor, acid and alkaline phosphatase, amylase, FRAP and uric acid.

Calcium and phosphor are the most abundant minerals in the human body, and are present in the saliva in ionized form and connected mainly to proteins. Their function is remineralization; therefore, they are involved in caries injuries and dental erosion (Devlim, 2011). In this study, patients with eating disorders presented a significant decrease in the levels of calcium and phosphor in their saliva when compared to the control group. However, in the study by Johansson, Norring, Unell and Johansson (2015), no difference was found between the groups.

The activity of phosphatase has already been evaluated in the saliva of several types of patients, such as diabetics (López et al., 2003), smokers (Prakash et al., 2016), women in post-menopause (Sophia, Suresh, Sudhakar, Jayakumar & Mathew, 2017), and healthy children (Chaves-Neto, Sassaki & Nakmune, 2011). The increase in the activity of these enzymes in the saliva has also been considered the consequence of the destructive process of the alveolar bone and tissue degradation in advanced stages of periodontal disease (Santos et al., 2018). In addition, the acid and alkaline phosphatases have been identified as indicators of the increase in the index of dental caries (Saito & Kizu 1959). We did not find randomized or case control clinical studies in the scientific literature that associated these enzymes with an increase in the indices of dental caries in patients with anorexia and bulimia, which justifies the present study. However, we did not find significant associations between these factors in this research.

Amylase is the most frequent secretion of the parotid gland, whose increased activity is supposedly the result of recurring vomits, particularly frequent in patients suffering from parotid swelling related to eating disorders (Scheutzel & Gerlach, 1991). The change in salivary composition can be attributed to the effect of a longer duration of the disease, which would probably have a more severe impact on the body's physiological functions (Johansson et al., 2015). However, no differences were found between the groups studied, a fact that corroborates the findings of Johansson et al. (2015). This finding may be due to the fact that patients with eating disorders belong to the mental health clinic and are undergoing regular treatment and follow-up, where it is presumed that the episodes of purging activities are restricted in many cases.

Regarding FRAP, other studies have correlated higher concentrations of total salivary protein with a higher total antioxidant capacity of the saliva in patients with carious lesions (Silva, Fraga, Costa, Dias & Brito, 2017); however, none of these studies analyzed patients with eating disorders. In this study, FRAP levels were higher in the case group, which had a higher index of dental caries.

Uric acid is also related to high DMFT levels in several populations (Silva et al., 2017), but not in individuals with anorexia and bulimia. This indication was found in this study, where the uric acid increase was related to the case group, which had a higher amount of teeth affected by caries disease.

5. Conclusion

In the patients with anorexia and bulimia, due to a decreased salivary flow that is characteristic of self-induced vomiting, there is an increase in the caries and dental erosion index when compared to the control group. Biochemical markers have proven to be auxiliary tools in the diagnosis of oral afflictions; however, by themselves, they cannot indicate the existence of diseases.

It is suggested that further work be carried out with salivary biochemical analyzes in women with anorexia and bulimia nervosa, so that more comparisons can be made between the results found.

Acknowledgments

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