**Macroscopic evaluation of the oral mucosa and analysis of salivary pH in patients with anorexia nervosa**

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**Summary**

The aim of the study was to evaluate the status of the oral mucosa, to assess the prevalence of Candida in the oral cavity and to analyze the pH values of total saliva in patients with anorexia nervosa (AN) in comparison to the general population.

**Method.** A controlled clinical trial was designed for two, age-matched, female groups: patients with AN (Group A, n=31) and healthy women (Group 0, n=40). Total saliva was collected at rest and after stimulation by chewing paraffin wax. Salivary pH was measured and macroscopic evaluation of the oral mucosa was performed with a qualitative and quantitative mycological analysis. The smear layer was collected from three different areas in the oral cavity. Selected Candida broths were used for incubation.

**Results.** Changes in the macroscopic structure of the oral mucosa due to multifactorial etiologies were observed. The prevalence of Candida in patients with AN was comparable to that in the general population. Salivary pH values were significantly lower in the AN patients than in the control group.

**Conclusions.** The incidence of pathological changes in the oral mucosa is associated with the loss of the salivary protective barrier. This is shown by the significant reduction in the pH values of stimulated and non-stimulated saliva of the AN patients. In these patients, the monitoring of salivary parameters such as salivary flow rate and pH is indicated, and a regular dental checkup, together with soft tissue evaluation, is advised.

**Key words:** anorexia nervosa, oral cavity
Introduction

Although eating disorders have probably always affected mankind, anorexia nervosa (AN) is a regarded disease of a modern culture and prosperity because of its steadily increasing incidence in developed countries [1, 2]. The main symptoms of the disease are a persistent quest for weight loss and a fear of weight gain. According to the American Psychiatric Association, the main criteria for the diagnosis of AN include a refusal to maintain body weight at a minimum normal weight for age and height (a reduction in body weight below 85% of that expected for age and height, a failure to achieve expected growth and a BMI – (body mass index) below 17.5), a distorted body image, amenorrhea for at least the three months and avoiding eating [3]. Depending on the duration and severity of the disease, eating disorders may secondarily lead to the development of multi-organ complications which include abnormal heart rhythm and blood pressure, anemia, dehydration, electrolyte imbalance, and hormonal damage to the lining of the gastrointestinal tract and bowel paralytic ileuses [4, 5].

The disease can lead to the development of defects in the oral cavity. Abnormalities can be observed in both the hard and soft tissues [6-8]. Pathological changes to the teeth, periodontium and mouth in general are related to, among other factors, malfunction of the major and minor salivary glands, shortages of micro- and macronutrients in the body, an imbalance between physiological and pathological flora in the oral cavity, and variations in pH, as well as in various compensatory behaviors adopted by patients during the course of the disease [9]. An increased incidence of non-carious cavities, erosion, abrasion and attrition, plus enamel and dentin hypersensitivity, and increased susceptibility to dental caries have all been reported in patients with AN [6-8]. The symptoms of the condition may include hyperplasia and edema of the small and large salivary glands, xerostomia and loss of taste, traumatic redness of the oral mucosa, atrophic inflammation of the tongue, angular cheilitis, oral candidiasis and burning mouth syndrome [10-12]. Despite multi-specialist research on AN problems affecting the oral cavity of these patients are still not properly addressed and require further analysis.

Salivary studies may identify markers of oral signs of eating disorders and may contribute to the early detection of the disease by dentists and doctors. This can help to avoid the adverse effects of some patient compensatory behaviors [13-15].

The aim of the current study was to evaluate the condition of the oral mucosa, incidence of Candida species in the oral cavity and salivary pH changes in patients with AN and to compare then with those in a group of people without eating disorders (Hypothesis 1). The null hypothesis was that there are no differences between the groups.

Material and Method

A controlled clinical trial was conducted at the University of Medical Sciences in Poznan in the years 2011-2013. The subjects were divided into two groups: A and 0.
Group A consisted of 31 patients with a diagnosis of anorexia nervosa confirmed by two independent psychiatrists from the Department of Child and Adolescent Psychiatry using the following codes ICD 10 (code F 50.0) and DSM -IV (code 307.1 ), [3]. The average age in this group was 15 ± 2 years, with the eating disorders having started 1.2 ± 0.3 years earlier.

The control group 0 consisted of patients receiving routine conservative procedures in the Department of Biomaterials and Experimental Dentistry and consisted of 40 healthy women who were matched for age (mean age 14 ± 1 years, Mann-Whitney test p > 0.05) and sex (females only) and without any past history of eating disorders. Other criteria for inclusion in the investigation were good general health, absence of pregnancy, and the presence of written informed consent to participate in the study. Exclusion criteria for both groups included active carious lesions, smoking, periodontal disease, systemic diseases and use of drugs that affect the secretion of saliva.

To avoid all the factors affecting the rate of flow and the pH of saliva, which might disturb the comparison between groups, all the participants were examined at the dental clinic at the same time of year (fall / winter), and in the morning between 9.00 and 12.00 am to minimize diurnal variation. Data were collected by the same person, a dentist. In preparation for the collection of saliva, each patient was informed that she should refrain from eating, drinking and regular oral hygiene procedures for at least 60 minutes before the test. Saliva was collected under both unstimulated and stimulated conditions: at rest for 15 minutes and then stimulated by chewing wax tablets for 5 min. The salivary pH measurements and analyses were performed within 2 hours of collection and were determined by potentiometry, using a fully automatic TM ABL 520 acid-base balance analyzer (Radiometer Medical A / S, Copenhagen, Denmark). This device uses an advanced system of non-contact electrodes to measure pH, pCO₂, pO₂ and a K606 calomel electrode for retaining a constant reference to the pH electrode. The ABL520 analyzer provides 7 types of calibration, 5 of which are carried out automatically.

The clinical part of the study was carried out in the Department of Biomaterials and Experimental Dentistry. All the clinical procedures were performed by a single investigator. The study was conducted according to the Good Clinical Practice guidelines and the pattern of the Declaration of Helsinki, after approval by the Bioethics Committee of the University of Medical Sciences in Poznan ( no consent: Resolution No. 719/11 and No. 64/12 ). The nature of experiment was explained to all of 71 participants, and were given a written informed consent to participate in the study.

As part of the study of the oral mucosa, subjective symptoms included duration of their occurrence, family history, habits, and current treatment. Macroscopic evaluation of the mucosa was performed using a standard dental diagnostic kit (mirror, probe, ENT spatula) in artificial light. All observed lesions were documented, recorded photographically and performed for further qualitative and quantitative analysis of mycological cultures. Swabs were taken from three different regions
of the oral cavity: palate, cheek and dorsal surface of tongue. The culture was grown on selective media for Candida species at 37 ° C. The results were recorded after 14, 48, 72 hours and 7 days.

The statistical analyses used the t-Student test for independent variables and the nonparametric equivalent of the above test – Mann-Whitney test. Statistical hypothesis was verified on the level of significance set at α=0.05.

Results

Group A. The subjective symptoms experienced by those with AN included a burning sensation of the oral mucosa in 4 subjects (12.9%). An objective analysis of the oral mucosa revealed the following pathological changes: *exfoliate cheilitis* (13 subjects; 41.9%), pallor of the oral mucosa and skin (9 subjects; 29.0%), *atrophic glossitis* (8 subjects; 25.8%), white coating of the tongue (8 subjects; 25.8%), *linea alba* (6 subjects; 19.3%), erethematous spots on the palate (4 subjects; 12.9%), *morsicatio buccarum* (4 subjects; 12.9%), *angular cheilitis* (4 subjects; 12.9%) and ulcers of a traumatic etiology (4 subjects; 12.9%). The presence of Candidal fungi (*type C. albicans*) was verified in 4 individuals with AN (12.9%), (Fig. 1, 2, 3, 4).

Group 0. In the control group not a single individual experienced subjective symptoms in the oral cavity. However, among pathological changes of the oral mucosa observed during an objective examination were; white coated tongue (10 subjects; 25.0%), *linea alba* (9 subjects; 22.5%) and geographic tongue (1 individual; 2.5%). The presence of Candidal (*type C. albicans*) organisms in the oral cavity was verified in 7 individuals within this control group (17.5%) which was not a statistically significant difference from the presence in Group A.

Analysis of unstimulated whole saliva revealed that its pH was lower in group A (6.6 ± 0.3) and that it differed statistically from that in the healthy individuals in group O (6.8± 0.2), (p = 0.0001). After masticatory stimulation both groups showed a difference in the pH of the unstimulated and stimulated whole saliva with Group A (7.1± 0.1) and Group O (7.2± 0.16). The level of statistical difference in pH between the groups was (p = 0.0011), (Table 1).

| Tab. 1 Differences between anorexic and healthy subjects. 
<table>
<thead>
<tr>
<th>Basic statistics – mean, {median}, range, (SD-Standard Deviation)</th>
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<tr>
<td><strong>Group A</strong> (n=31)</td>
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<tr>
<td><strong>Age [years]</strong></td>
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<td><strong>Height [cm]</strong></td>
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A number of pathological changes to the oral mucosa were observed in the patient suffering from AN. The only subjective symptom experienced by patient in group A were a burning mouth sensation, which was reported by 4 individuals (12.9%). Johansson et al reported this symptom more frequently in anorexic patients than in the control group [16]. Some of the factors that predispose people with AN to burning mouth syndrome include: xerostomia, deficiencies in macro and micro elements, irregular eating, eating at night, vomiting, large daily fluctuations in the pH of the oral cavity, mechanical and chemical irritation of the oral mucosa, hormonal imbalances experienced during eating disturbances, side effects of certain drugs (antidepressants, diuretics, laxatives), as well as the nervous states and stresses that accompany the basic illness (anorexia nervosa) [8, 17].

The most frequently pathological change observed within the anorexic group was exfoliative cheilitis, which was observed in 41.9% of individuals. Johansson et al [16] also observed a greater frequency of drying and cracking of the oral mucosa around the lips much more frequently in patients with anorexia than in healthy individuals. Exfoliative cheilitis in individuals suffering from anorexia nervosa is probably caused by, among other things, dehydration and decreased salivary secretion, deficiencies of microelements (e.g., vitamins A and B) associated with bouts of starvation, as well as parafunctions arising from stress (habitual lip biting). Other types of keratosis: linea alba and morsicatio buccarum associated with habitual biting of the mucus membrane were observed in 10 individuals in our experiment (32.2%). Both types
Fig 1. *Angular cheilitis, exfoliative cheilitis and skin pallor in an 18 year old patient (S.O.)*.

Fig 2. *Exfoliative cheilitis with a crack in the lip midline and pallor in a 14 year old patient (M.S.)*.
Fig 3. Erythematous spots and erosions of the palate of a 14 year old patient (A.K.).

Fig 4. Excessive keratinization of the oral mucosa caused by habitual cheek biting in a 14 year old patient (K.J.).
of keratosis are localized on the mucus membrane of the cheeks and their appearance is evidence of habitual pressing or biting of the soft tissues, which is usually a reaction to strong and long term stress. A similar causal mechanism results in the appearance of erosions of traumatic etiology, found in 12.9% of our Group A individuals. Erosions and ulcers of the oral mucosa can arise in patients with eating disorders and be caused by various types of compensatory actions, mainly ones which arise during the mechanical stimulation of the back of the throat by individuals seeking to provoke vomiting [18, 19]. Pallor of the skin and oral mucosa was observed in 29% of Group A individuals, which could suggest the possibility of an increased occurrence of anemia in anorexic individuals. Another symptom of anemia seen in these subjects and associated with iron deficiency (microcytic) or vitamin B₁₂ deficiency (megaloblastic) was also erosive glossitis, seen in 25.8% of the subjects in group A. Classical symptoms of iron deficiency anemia are smoothing of the tongue and disappearance of papilla. Deficiencies of group B vitamins have an inhibitory effect on the epithelial cell cycle, which leads to epithelial thinning and disappearance [11]. A different variety of anemia related to Vitamin B₁₂ deficiency is associated with characteristic erosive alterations in an inflamed tongue and is called Hunter’s tongue, in which alternating white and red streaks appear on the dorsum of the tongue after it is protruded from the mouth.

The presence of the white sediment on the tongue of 25.8% individuals pointed to certain hygienic deficiencies. The results obtained from epidemiological studies relating to the oral hygiene habits of patients with AN are equivocal. Daszkowska et al. and Shaugnessy et al. did not observe any important differences in oral hygiene between patients with AN and their control group while Yagi maintained that along with the progression of AN there did appear to be a significant worsening in oral hygiene [20-22]. An increase in the amount of deposits on the tongue we observed may have been caused by a decrease in salivary flow. The erethematous spots found on the palate of 12.9% of anorexic individuals, and the similar percentage of traumatic erosions, may have been caused by mechanical and chemical irritation and indicate atrophic processes in the mucous membrane of the oral cavity as suggested by Paradowska (2010) and Misra (2010), [11, 18]. The angular cheilitis found in 12.9% of our AN individual suggests the possibility of coexisting deficiencies of microelements (mainly vitamins from groups A and B, as well as of iron) and a mixed bacterial-fungal infection [23]. An increased susceptibility and risk of candidiasis in AN individuals is caused by, among other things: disturbance of the immune system resulting from malnutrition, stress and nervous and anxiety states, a decrease in salivary flow, thinning and atrophy of the oral mucosa caused by a deficiency in microelements and significant disturbances in the pH of the oral cavity, which result from irregular eating habits. Our observations did not reveal an increase in frequency of the appearance of fungi in the oral cavity. Candidal fungi were only found in 12.9% of our AN subjects. Back-Brito et al (2012) obtained very different results because, in their study, it was found that
people with eating disorders had an increased presence and a higher differentiation of these same fungi in the oral cavity than healthy controls [24].

Salivary pH changes

Our results confirm the opinion of other authors that patients suffering from AN manifest sialochemical disturbances which appear as a low salivary pH. This can be caused by a decrease in buffering elements, which are dependent on the speed of salivary flow. Saliva and its buffering elements protect the environment of the oral cavity from fluctuations in pH [25]. The excretory capacities of the salivary glands play a crucial role in maintaining the functions of saliva (defensive, lubricatory, wetting, buffering, and related to the integrity of the oral mucosa). Parotid salivary glands are responsible for secreting the inorganic elements of saliva and maintaining a neutral pH. The pH value of saliva as an extracellular fluid is maintained within the range of 5.0 – 7.0. This considerable variability in salivary pH indicates saliva’s role in maintaining an acid-base balance within the oral cavity, as well as its large buffering capacity. The available literature points to the fact that salivary pH is determined by bicarbonate ions as part of the carbon/bicarbonate buffering system and by phosphate ions (PO₄³⁻) as part of the phosphoric/hydrogen buffering system and, to a lesser degree, by peptides such as urea [26, 27]. Bicarbonate and phosphate ions are undoubtedly the most important in maintaining osmolarity and buffering equilibrium. It is known that the concentration of bicarbonate ions is directly proportional to the amount of saliva secreted by the salivary glands. Insufficient production of saliva leads to a deficiency in factors that are important in maintaining a safe pH within the oral cavity. According to Little, differences in salivary pH can be caused by elevated levels of bicarbonate, which reduce the concentration of free bicarbonate ions, and not just by a reduction in salivary flow [28]. The higher the salivary ion concentration of HCO₃⁻ the higher will be the pH. HCO₃⁻ anions are the main buffers in stimulated saliva because their concentration increases by 40-60mmol/L. This is mainly due to the contribution of the parotid glands directly with an increase in salivary flow, while in the salivary resting state the bicarbonate ion concentration is low (only about 1mmol/L), [26, 27]. Consequently, in individuals with lower levels of salivary secretion (xerostomia) there is a disturbance in the supply of HCO₃⁻ ions and a corresponding decline in both the pH and in the buffering capacity of saliva. Phosphate ions are less significant in maintaining the pH of the oral cavity. Their concentration is highest in unstimulated saliva (about 10-20mmol/L). The rapid secretion of unstimulated saliva is correlated with a decrease in inorganic phosphate ion concentration and an increase in pH, resulting in a decreased intensity of the acid-producing processes. A slow-down in salivary secretion is correlated with a drop in pH and an increase in inorganic ion concentration. Most authors emphasize the influence of starvation on the loss of fluids and in the dehydration of the organism, which can manifest themselves as decreased output of the salivary glands [8,29]. This type
of experience was created experimentally by depriving healthy individuals of food and water for 24 hours. In addition to metabolic symptoms of dehydration, such as a decrease in hematocrit and hemoglobin levels, a decrease in sodium, protein and creatinine levels in the urine and in urine osmolality, a reduction in parotid gland output was also observed [30]. When considering the length of time that individuals in group A were ill (the average time span of the illness was only 1.2 years), it seems that the changes we observed in the oral mucosa and the decrease in oral cavity pH may testify to a loss of protection to the oral mucosa resulting from reduced salivary gland activity.

Conclusions

Amongst patients suffering from AN macroscopic changes in the oral mucosa were observed due to multifactorial causes: traumatic, inflammatory, stresogenic, and resulting from deficiencies in food and dehydration. The progression of these types of damage appear to be directly related to the loss of protective ability of saliva. This is indicated by the decreased pH value in both unstimulated and stimulated whole saliva. The presence of pathological changes in the oral mucosa of these anorexia nervosa patients indicates the necessity of controlling their salivary parameters such as salivary flow rate and pH, and the need for a regularly scheduled dental appraisal of the state of the soft tissues of the oral cavity.
References


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