Parotid salivary parameters in bulimic patients – a controlled clinical trial

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Summary

Aim. The aim of this study was to determine whether patients with purging-type bulimia and/or non-bulimic patients, treated with serotonin reuptake inhibitor SI-5-HT (fluoxetine), have dental erosion and changes in selected buffer components of parotid saliva (bicarbonates, phosphates, urea), compared with the healthy population.

Material and method. A controlled clinical trial was designed for three, age-matched, female groups of 94 patients: 1) bulimic patients treated with fluoxetine 40mg/day (n = 25), 2) non-bulimic patients diagnosed with bipolar affective disorder, treated with fluoxetine 20mg/day (n = 25), and 3) healthy controls (n = 44). Parotid saliva was collected from the subjects by means of Lashley cup at rest and stimulated chemically with a 3% citric acid solution. In clinical examination the dental erosion was determined as non-curious tooth substance loss using the Tooth Wear Index (TWI). The concentrations of inorganic phosphates, bicarbonate, urea and pH in saliva were measured.

Results. In the bulimic subjects higher TWI (24%) and lower levels of pH, bicarbonates and phosphates compared with controls were observed. There were no significant differences in urea concentration.

Conclusions. Erosive-abrasive tooth surface loss seems to be a significant diagnostic tool of bulimia nervosa. The presence of pathological changes in teeth structure indicates the loss of protective properties of saliva, which is proved by pH value and concentration of buffer ions. It is advisable to monitor salivary parameters, such as salivary flow rate, pH and the

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concentration of buffer ions in long-term treatment with SI-5-HT drugs in case of patients with purging-type bulimia. There is also a need for regular dental check-ups of the oral cavity tissues.

**Keywords**: bulimia nervosa, saliva

### Introduction

There is a number of diseases with chronic gastric regurgitation. The most common are psychosomatic complaints, such as alcoholism, eating disorders, and gastro-oesophageal reflux disease (GERD) \[1\]. Anorexia nervosa (AN) and bulimia nervosa (BN) affect 1–3% of young women \[2–5\]. Epidemiological studies on the incidence of bulimia nervosa, conducted in the United States and Europe, have shown an increase over the last 40 years \[6\].

Chronic regurgitation of acidic gastric contents can cause clinical changes in the oral cavity including dental erosion and other oral effects: tooth hypersensitivity, loss of vertical dimension, anterior open bite, bilateral parotid enlargement and xerostomia (dry mouth) \[7–10\].

One of the main factors which protects the oral environment against a fall in oral pH levels is saliva and its buffering capability \[11–13\]. The secretory capacity of salivary glands plays a basic role in the maintenance of all the functions attributed to saliva. The parotid gland is mainly responsible for the secretion of inorganic constituents of saliva and the maintenance of a neutral pH level. In unstimulated saliva, about 25% of salivary fluid is derived from the parotid gland but, under stimulation, this contribution increases by up to 50%. The maintenance of a near neutral pH, together with the number of calcium ions (Ca\(^{2+}\)), trivalent phosphate (PO\(_4^{3-}\)), bicarbonate (HCO\(_3^-\)) and urea are the most important factors in the buffering ability of saliva, the solubility of enamel hydroxyapatites, and its protective properties against erosion. A reduction in mineral components of parotid saliva may diminish or destroy these properties \[12, 13\].

### Aim

The purpose of this study was:

1. Determining whether vomiting bulimic and/or non-bulimic depressive patients, treated with the serotonin reuptake inhibitor SI-5-HT (fluoxetine), exhibit changes in their hard tissue (erosion) and parotid salivary inorganic components: bicarbonates, phosphates and nitrogenous products, such as urea.
2. Determining whether there is an effect of drug therapy on the examined parameters of salivary gland.

The null hypothesis was that there are no differences between the groups.

### Material and Method

This clinical controlled study was conducted at Poznan University of Medical Sciences and designed for the following three groups of female subjects.
Bulimic medicated group (group X) consisted of 25 female subjects diagnosed with bulimia nervosa by two independent psychiatrists (AS and AR) from the Department of Child and Adolescent Psychiatry of Poznan University of Medical Sciences, according to ICD-10 (code F 50.2) and DSM-IV (code 307.51) [14]. The frequency of vomiting was at least once per day and the mean frequency of vomiting was 1.9 ± 0.4/day. The mean age in this group was 21.2 ± 3.2 years, with a mean onset of eating disorder having occurred for 3.5 ± 2.4 years. Each patient had been receiving treatment with fluoxetine for 3.0 ± 1.8 months at a dosage of 40mg/day taken in the morning.

Non-bulimic medicated group (group Y) consisted of 25 non-bulimic female patients examined and diagnosed with bipolar affective disorder by the same two independent psychiatrists (child and adolescent psychiatrist (AS), also representing a specialization in adult psychiatry and adult psychiatrist (AR)), according to ICD-10 (code F 32.0) and DSM-IV (code 296.21) [14]. The subjects were not currently hospitalized at the Department of Child and Adolescent Psychiatry, but some of them suffered since the period of adolescence and has been hospitalized in that clinic in the past. Patients diagnosed with bipolar disorder received primary treatment course (besides fluoxetine) with mood stabilizers: valproic acid (dose of 800 to 1500 mg) or carbamazepine (dose of 800 to 1200 mg). None of the subjects received lithium.

The group Y was homogenous according to age (27.4 ± 6.3 years, Kruskal-Wallis test p > 0.05) and gender (only women) and the subjects were treated with the same medication, fluoxetine, for 4.4 ± 3.1 months at a dose of 20mg/day, taken in the morning. All the subjects in this group denied any history of eating disorders, additional disease or medication affecting salivation. Other criteria of inclusion in the study included good health status, not being pregnant and written informed consent. Exclusion criteria were active carious lesions, tobacco use, periodontitis, serious diseases and medication affecting salivation.

Control group (group Z) was recruited from dental patients attending the Department of Biomaterials and Experimental Dentistry and included 44 healthy female controls who were matched according to age (25.5 ± 4.6 years, Kruskal-Wallis test p > 0.05) and gender (only women). All subjects denied any history of eating disorders or bipolar affective disorder. Inclusion criteria were: age, gender, good health condition, not being pregnant, no medications (birth control agents excluded) and dietary supplements being taken. Exclusion criteria were: active carious lesions, tobacco use, periodontitis, chronic diseases and medication affecting salivation. The procedure of inclusion to/exclusion from the study was carried out by two dentists on the basis of subjective and objective tests (EP, MDK). Criteria of patient selection for groups X, Y, Z are shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vomiting</th>
<th>Fluoxetine (SI-5-HT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group X</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Group Y</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Group Z</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ present factor; – factor not present
During the clinical examination the teeth surfaces loss was assessed using the Tooth Wear Index (TWI), [15–17].

Unstimulated and stimulated saliva was collected according to the recommendations of Birkhed and Heintze [18] and Fontana et al. [19]. To avoid all factors affecting the salivary flow rate and pH, the patients were examined in the dental clinic during the same time of year (autumn/winter) and in the morning between the 9.00 and 12.00, to minimize circadian variations. The whole procedure of clinical assessment and collecting biological material of a given patient was performed by the same dentist (EP). During preparation for saliva collection, each subject was advised to abstain from eating, drinking and normal oral hygiene procedures for at least 60 minutes before the examination. Parotid saliva was collected under unstimulated and stimulated conditions. Parotid saliva was collected by placing a Lashley cup over the Stensen’s duct under different salivary flow conditions: at rest for 15 min and stimulated by the application of 3% citric acid. Estimation of the pH and bicarbonate ion analysis were performed within 2 hours after saliva collection using potentiometric method with a fully automated analyzer of acid-base balance ABL TM 520 (Radiometer Medical A/S, Copenhagen, Denmark).

The concentrations of inorganic phosphates and urea were measured using spectrophotometric and enzymatic methods with a Delta Beckman Synchon CX7 chemistry analyzer (Beckman Coulter Inc., Germany) and the Randox reagents kit (Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, UK).

All the clinical procedures were performed in dentist’s office. The study conformed to the Declaration of Helsinki and was performed according to the guidelines of the Good Clinical Practice. The ethical committee of Poznan University of Medical Sciences granted its approval for this study (Resolution No. 829/01). The nature of the dental experiment was explained to all 94 participants, all of whom gave their written consent.

For the statistical analyses, the nonparametric equivalents to analysis of variance (ANOVA) and the Dunn’s Kruskal-Wallis analysis of ranks (all pairwise multiple comparison procedures) were used. A significance level was set at α = 0.05. To express a relationship between two variables Spearman’s rank correlation coefficients were used (where $r_s = 0$ means no correlation, $r < 0.3$ poor correlation, 0.3 to 0.5 fair correlation, 0.6 to 0.8 moderately strong and over 0.8 very strong correlation).

**Results**

The results showed that there were statistically significant differences in tooth wear (TWI) between bulimic group X (24%) and group Y (10%) and control group Z (9%). The total loss of enamel and dentin (more than ½ of its thickness) with a value of TWI = 3 was observed only in the group X of patients with bulimia. In any group there were no tooth wear with the dental pulp exposure (TWI = 4). Analysis of the unstimulated parotid salivary constituents demonstrated that the pH and bicarbonate levels were the lowest in bulimic group X ($p \leq 0.05$). There were no significant differences in phosphate ($p = 0.0525$) and urea ($p = 0.0564$) concentrations amongst the separate groups (Table 2).
Table 2. Analysis of parameters of unstimulated parotid salivary in groups X, Y, Z

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group X n = 25</th>
<th>Group Y n = 25</th>
<th>Group Z n = 44</th>
<th>Kruskal-Wallis test</th>
<th>Dunn’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.18 (0.77)</td>
<td>7.48 (0.54)</td>
<td>7.59 (0.47)</td>
<td>0.0385* X vs. Z 0.0337*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.30–8.18</td>
<td>5.87–8.07</td>
<td>6.12–8.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>6.1 (6.1)</td>
<td>11.9 (9.6)</td>
<td>12.3 (11.4)</td>
<td>0.0246* X vs. Z 0.0459*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0–24.2</td>
<td>0.1–33.2</td>
<td>0.2–52.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>20.1 (10.4)</td>
<td>14.8 (7.5)</td>
<td>14.5 (4.9)</td>
<td>0.0525 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.9–42.5</td>
<td>9.0–45.3</td>
<td>5.4–26.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>33 (13)</td>
<td>36 (10)</td>
<td>29 (10)</td>
<td>0.0564 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8–59</td>
<td>20–60</td>
<td>12–55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group X – bulimic patients receiving 40mg fluoxetine/day; Group Y – non-bulimic patients receiving 20 mg fluoxetine/day; Group Z – healthy controls; * statistically significant differences

After chemical stimulation, all the groups revealed pH level differences between unstimulated and chemically stimulated parotid saliva. However, there were no significant differences in pH amongst the separate groups (p = 0.0970). The pH and bicarbonate rates were the lowest in bulimic group X (p ≤ 0.05). The bicarbonate ions concentration differed statistically, when compared to the results from the individuals from group Z (p = 0.0321) and Y (p = 0.0185). The phosphate and urea concentrations of stimulated saliva in all groups decreased when compared to the unstimulated rates. In regard to phosphate levels, differences appeared between groups X and Y (p ≤ 0.0396), and Z (p ≤ 0.0098). Similar downward trend after stimulation was observed in the case of urea, but with no differences in group X. However, there was a significant difference in the case of increased values of urea concentration for group Y (p ≤ 0.0291; p ≤ 0.0129). The data are presented in Table 3.

Table 3. Analysis of parotid salivary parameters after stimulation in groups X, Y, Z

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group X n = 25</th>
<th>Group Y n = 25</th>
<th>Group Z n = 44</th>
<th>Kruskal Wallis test</th>
<th>Dunn’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.11 (0.73)</td>
<td>7.32 (0.42)</td>
<td>7.39 (0.33)</td>
<td>0.0970 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.07–8.18</td>
<td>6.25–7.86</td>
<td>6.86–8.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>9.9 (8.9)</td>
<td>18.1 (11.3)</td>
<td>16.1 (10.1)</td>
<td>0.0112* X vs. Z 0.0321*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0–31.7</td>
<td>3.1–41.7</td>
<td>0.0–41.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>17.9 (10.1)</td>
<td>11.6 (4.5)</td>
<td>11.1 (4.2)</td>
<td>0.0080** X vs. Y 0.0098**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6–39.5</td>
<td>5.6–23.6</td>
<td>2.3–20.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>27 (11)</td>
<td>35 (8)</td>
<td>27 (11)</td>
<td>0.0083** X vs. Y 0.0129**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10–54</td>
<td>21–57</td>
<td>7–50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group X – bulimic patients receiving 40mg fluoxetine/day; Group Y – non-bulimic patients receiving 20 mg fluoxetine/day; Group Z – healthy controls; *, ** – statistically significant differences
In all groups the highest Spearman’s rank correlation coefficient (0.7 ≥ r < 0.9) was between pH and bicarbonate ions (p ≤ 0.0001). There was also a high dependency (0.3 ≥ r < 0.5) between pH levels and phosphate ions in group Y and Z (p ≤ 0.003). This correlation was not present in group X (p = 2.436).

Discussion

In patients with purging type of bulimia, different signs may occur in the oral cavity, and our results showed that there were significant differences in the tooth wear between bulimics (group X) and non-bulimics (groups Y, Z) [20]. The results obtained from this investigation confirm the other authors’ opinion that there are sialochemical disturbances in patients suffering from bulimia, which are manifested by low pH level. Analysis of salivary buffer components showed that they were significantly reduced in bulimic patients (group X) after chemical stimulation of salivary secretion. Similar results concerning the levels of fluctuations in inorganic components have been obtained by other authors [21–24]. The reason for the buffering disturbances are explained as being a result of parotid dysfunction [25], the use of antidepressants [26–28], or probably a combination of these two factors.

In patients from group X the supply of bicarbonate buffering ability of saliva was lower in bulimic group. It is believed that at higher flows of stimulated secretion, the concentration of bicarbonate ions is higher [13]. Insufficient saliva production leads to a deficiency of factors maintaining a safe, neutral pH in the oral cavity. Therefore, bicarbonate ions, under stimulated conditions, are responsible for most of the buffering capacity of saliva. The low bicarbonates level will act as a co-factor in erosion cases. However, in the literature related to those bulimic patients who indulge in self-induced vomiting, no direct linear relationship was found between vomiting experience and erosion. Numerous studies have shown inconsistent results regarding the salivary parameters such as pH, buffering capacity and flow rate. Milosevic and Dawson [29] indicated that above a threshold of 1,100 vomiting episodes (from 3 to 5 years of history of bulimia) chemical dissolution of dental tissue as erosion is highly probable. However, this the issue of how many vomiting episodes may cause a pathological erosion is still under discussion.

The inorganic orthophosphates in saliva consist of phosphoric acid H₃PO₄²⁻, primary H₂PO₄⁻ secondary HPO₄²⁻ and tertiary PO₄³⁻ inorganic phosphate ions. All of them are responsible for buffering capacity and the maintenance of a neutral pH level in unstimulated saliva. A high concentration of these phosphates enables neutralization of dental tissues by ionic exchanges directed towards the tooth surface. Their concentration depends upon the salivary secretion level and the pH level. It is believed that the maximum buffering capacity of the phosphates is correlated to the pK and a pH of 6.8–7.2. When salivary secretion increases, the total concentration of inorganic phosphates decreases and acid-forming processes are less severe [13]. This relation was observed in our study in all groups with the exception of the bulimic group X. The elevated concentration of phosphate ions in the group X may suggest a reduction in salivary gland secretion. Based on the phosphate ion changes in concentration
found before, and after, salivary gland stimulation it seems that their buffering role in bulimic patients was insufficient. Urea concentration was the third buffering system assessed in our investigation. There were no significant differences in urea concentration between patients from groups X, Y and Z. The concentration of urea is low (only 1/13 of the plasma proteins) and is independent of the rate of salivary secretion, but dependent on the concentration in the blood [30]. This probably was the cause of the lack of differences in the urea concentration between patients from groups X and groups Y and Z, that despite impaired saliva secretion urea concentration has not changed. The stability of urea concentration can also express a partial adaptation of the body to a state of permanent vomiting.

Another cause for sialochemical disturbances might be the influence of anti-depressant medications on those receptors and neurotransmitters in the central nervous system which are responsible for salivary gland stimulation and indirect pH maintenance. The anti-cholinergic effects of tricyclic and tetracyclic anti-depressants are well documented. These medications block the M muscarinic receptors, or release noradrenaline. The pharmacological effect of anticonvulsant drugs (valproic acid and carbamazepine) is associated with stimulation of the alpha 2 adrenergic receptors and increasing concentration of gamma-aminobutyric acid (GABA) in the central nervous system [31].

It is well known that salivary secretion is under the control of the autonomic nervous system. Parasympathetic nerves release acetylcholine (ACh) and stimulate fluid secretion; sympathetic nerves release noradrenaline (NA) to induce protein production [32]. There is a chain of reactions from binding of muscarinic, α – and β-adrenergic receptors to a plasma membrane of secretory cells of the salivary glands and thus giving the signal to the synthesis and secretion of water, electrolytes and proteins. Any failure in neural transmission or increase of noradrenaline in patients with bulimia could induce a reduction in the production of saliva [33].

In clinical trials of various anti-depressants, salivary gland output was reduced after 14 days of treatment with a minimal dose of 50 mg, or after a one-time dose of 75 mg [34–36]. In addition to receiving anti-depressants, bulimic patients are frequently treated with selective serotonin re-uptake inhibitors, one of which is fluoxetine [37, 38]. Fluoxetine is prescribed in the treatment of bulimia nervosa in doses between 40 and 80 mg (average 60 mg) and in depression at doses of 20–40 mg [36]. In our study, the daily dose of SI-5-HT in the group X was 40 mg, and in group Y – 20 mg. The differences in doses between the two groups X and Y in this study and their impact on the results must be taken into account, as they might influence the smaller changes in the group Y. In most of the research papers comparing the various medications used in treating bulimic patients, i.e. tricyclic and tetracyclic anti-depressants and selective serotonin re-uptake inhibitors, it was shown that the latter group of medications impaired salivary secretion to a much lesser extent than the former [27, 34, 35]. We found that the parotid concentration of buffers was not reduced enough in our bulimic and non-bulimic medicated subjects to cause severe acid-base disturbances when compared to the healthy controls. Therefore, it seems that medications from the SI-5-HT group (selective serotonin reuptake inhibitors), including fluoxetine, which have been proven
to be therapeutically effective, may be used to treat bulimic patients without the fear of salivary alteration. After a long-term use of these drugs, it is advisable to monitor salivary parameters, such as the rate of salivary secretion, pH and the concentration of buffer ions. In this way it is possible to prevent erosion and changes of the oral mucosa. The locating of defects in the oral cavity during the physical examination allows clinicians to determine the cause and duration of eating disorder.

Conclusions

Erosive-abrasive tooth surface loss seems to be a significant diagnostic tool in bulimia nervosa. The presence of pathological changes in teeth structure of bulimic patients indicates the loss of protective salivary properties such as salivary flow, pH and concentration of buffer ions. It is advisable to monitor salivary parameters, such as pH and the concentration of buffer ions after long-term use of medications from the SI-5-HT group in case of purging bulimic patients. There is also the need for regular dental check-ups of the oral cavity tissues.

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Figure 2. Erosion of the buccal surface of the lower premolars in a patient aged 29

Figure 3. Attrition of incisal edges of upper front teeth in a patient aged 19
Figure 4. **Bilateral parotid gland swelling in a patient aged 20**

References


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