Periodontal disease, oral microbial flora and salivary antibacterial factors in diabetes mellitus type 1 patients

G. Pinducci¹, L. Micheletti², V. Piras², C. Songini³, C. Serra⁴, R. Pompei⁴ & L. Pintus¹
¹ Dipartimento di Igiene e Sanità Pubblica, Università di Cagliari; ² Istituto di Odontoiatria e Chirurgia Maxillo Facciale, Università di Cagliari; ³ CDMA, Ospedale G. Brotzu di Cagliari; ⁴ Istituto di Medicina Interna, Sezione di Microbiologia, Università di Cagliari, Italy

Accepted in revised form 25 March 1996

Abstract. One hundred and thirty-one patients with diabetes mellitus type 1 (IDDM) and 20 healthy controls were checked for the presence of periodontal diseases and for some oral microbiological parameters. Results demonstrated that IDDM patients, who were well compensated from both the metabolic and clinical point of view, showed a prevalence for periodontopathies, which only differed slightly from controls. Only the presence of gingivitis was significantly higher in IDDM patients than in healthy subjects. Both anaerobic and aerobic microbial flora did not show substantial differences for either group. Among the salivary antibacterial factors studied, lysozyme was significantly decreased in diabetic patients compared to controls. It is concluded that IDDM patients undergo periodontal complications with a frequency quite close to that of non-diabetic healthy subjects, when the disease is under strict metabolic and clinical control.

Key words: Antimicrobial factors, Diabetes mellitus type 1, Microbial flora, Periodontitis

Introduction

Insulin dependent diabetes mellitus type 1 (IDDM) is at present the most common chronic childhood endocrinometabolic disease [23]. The total frequency of the disease, (i.e. IDDM and non insulin-dependent diabetes mellitus, NIDDM), is reported to have increased in the last decade mostly in western countries [2]. In Italy, diabetes affects about 4–5% of the total population, mostly females (around 3 million patients on the whole with 10% insulin dependent), with a tendency that has increased from the sixties up to the present day [8, 12, 34]. Europeans (Caucasians) are the most at risk from IDDM among the populations of the world. Epidemiological studies on IDDM incidence have shown a decreasing gradient from Northern Europe to the Mediterranean regions. The EURODIAB study, while confirming this general rule of latitude, showed Sardinia as a striking exception, which, with its annual rate of 34/100,000 new cases, represents the second highest risk area for IDDM in Europe after Finland [13]. This incidence rate shows that males are slightly more affected by IDDM than females and tends to be generally higher in summer. This seems to be confirmed by a recent survey carried out among Sardinian conscripts examined over the last 35 years and simultaneously contradicts the ‘north-to-south gradient’ hypothesis [37].

Life prolongation through insulin therapy, with a consequent significant improvement in life-style, has eventually revealed a set of catastrophic long-term complications such as retinopathy, nephropathy, neuropathy, as well as premature mortality following cardiovascular diseases [7]. Among these complications, periodontopathy is generally reported to be very common among diabetics, as diabetes mellitus is one of those chronic diseases which can affect periodontal tissues [11, 19]. This is why many authors have started studying the relationship between periodontopathies and diabetes mellitus [9, 15, 17, 37, 39] either IDDM [3–6, 14, 16, 22, 26, 30, 32, 38] or NIDDM [10, 21]. Since different periodontal indexes have been used and different statistical analyses have been carried out, results are often contradictory [27, 36]: some authors are of the opinion that periodontopathies are more frequent in diabetics [14–16, 18, 23, 24, 26, 27, 30], while others do not think so [29, 30, 35].

All however seem to agree that the different degrees of severity of periodontopathy in diabetics should be ascribed to diabetic micro- and macroangiopathies, to modifications in the metabolism of collagen, to alterations of the oral and gingival flora and particularly to the composition of the bacterial plaque as well as to weakened local defence mechanisms [3, 21, 41].

Oral mucosa is colonized by several species of commensal microorganisms, which can occasionally participate in the pathological processes of dental and
periodontal tissues [24]. The factors that can induce these bacteria to switch from commensalism to pathogenicity have been widely studied [18]. Some bacteria produce virulence factors which are responsible for injuries to hard and soft oral tissues. This is the case of *Streptococcus mutans*, which produces polysaccharides (mutans) that allow it to stick to the teeth, thus permitting the enamel to be degraded by the lactic acid produced [20]. This is also the case for some *Bacteroides* species, which produce short-chain acids, that are able to induce inflammatory processes in periodontal tissues [28]. Nevertheless, more virulent strains can often be found in the oral cavity, without any evident damage to oral tissues [28]. This fact is due to several factors which modulate the pathogenicity of oral bacteria; among these, it is worth mentioning an intrinsic genetic resistance to oral pathogens, the production of antibacterial factors, such as lysozyme and acetyl-

**Subjects and methods**

**Patients.** Patients with IDDM (131: 70 males and 61 females, with age range between 5 and 65), and 20 non-diabetic apparently healthy comparable controls were studied. IDDM patients were all under intensive insulinic therapy (3-4 injections/day) and had a fair metabolic control (HHBA1c at a mean of 7.07).

The date of IDDM onset, as well as glycometabolic parameters, general vascular complications and periodontal indexes were recorded for all patients. Control subjects underwent a medical exam and their periodontal indexes were determined. The gingival index according to Loe (25), dental mobility and the periodontal index of community therapy needs (CPITN) were evaluated (1). The score of these indexes was read as indicated in Table 1.

**Table 1.** Mode of evaluation of some odontostomatologic parameters

<table>
<thead>
<tr>
<th>Value</th>
<th>Gingivitis</th>
<th>Dental mobility</th>
<th>CPITN index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absent</td>
<td>Absent</td>
<td>Negative</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>Mild</td>
<td>Bleeding</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Plaque, tartar and pockets &lt; 4 mm</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Severe</td>
<td>Pockets between 4 and 5.5 mm</td>
</tr>
<tr>
<td>4</td>
<td>_</td>
<td>_</td>
<td>Pockets &gt; 5.5 mm</td>
</tr>
</tbody>
</table>

*CPITN = index of periodontal need of community therapy.*

*The value 4 is not calculated for gingivitis and dental mobility.*

**Sample collection.** Oral hygiene was checked in all the subjects before performing the collection of specimens. A simplified Grenn-Vermillon index (OHI-S) was used. Six different teeth were examined for each patient, both in the vestibular and tongue side. Alveolar bone loss was also detected. An X-ray examination allowed the evaluation of crestal bone loss and, using a graduated sound, it was possible to detect the bone loss at the level of the enamel-cement junction.

Samples of supragingival dental plaque and of crevicular fluid were collected from each patient and from the controls. The plaque samples were taken by means of a sterile curette from three different teeth. Crevicular fluid was collected by inserting a sterile paper cone for 5 sec into the interdental gingiva of three different teeth. The samples taken from each subject were pooled and weighed, and approximately 3 mg of dental plaque and 3 mg of crevicular fluid were diluted in 1 ml of Schaedler broth (Difco). Samples of saliva were also collected in sterile falcon tubes and frozen at −20 °C until use. Within one hour the specimens were sent to the laboratory and processed for microbiological analyses. Cultures of supragingival plaque and of crevicular fluid were made by standardized microbiological methods as indicated elsewhere [33], using the following media: CLED agar, Columbia CNA blood agar, MacConkey agar, Schaedler agar and Sabouraud agar (all from Microbiol, Cagliari). The *Capnocytophaga* spp. were identified on trypticase blood bacitracin-polymixin agar (Bio-Merieux Italia).