Relationship between salivary melatonin levels and periodontal status in diabetic patients

Abstract: Among other functions, melatonin exerts both antioxidative and immunoregulatory roles. The indoleamine is secreted in the saliva, although its role into the mouth is not known. Diabetic patients frequently display oral cavity pathologies such as periodontal disease (PD), an inflammatory disease coursing with an increase in free radical production. Thus, we compared the degree of PD and interleukin-2 (IL-2) levels with melatonin concentrations in plasma and saliva of diabetic patients. A total of 43 diabetic patients (20 with type I and 23 with type II diabetes) and 20 age- and sex-matched controls were studied. Dental and medical history of all patients was in accordance with the criteria of the WHO. The periodontal status was evaluated by the Community Periodontal Index (CPI). Plasma and salivary melatonin levels were determined by specific commercial radioimmunoassays, and plasma IL-2 was measured using a commercial enzyme-linked immunosorbent assay kit. Diabetic patients had plasma and saliva melatonin levels of 8.98 ± 7.14 and 2.70 ± 2.04 pg/mL, respectively. These values were significantly lower (P < 0.001) than those obtained in plasma and saliva of controls (14.91 ± 4.75 and 4.35 ± 0.98 pg/mL, respectively). Plasma and salivary melatonin concentrations show a biphasic response in diabetic patients. Melatonin decreased in patients with a CPI index of 2, and then increased reaching highest levels in patients with a CPI index of 4. By contrast, IL-2 levels decreased from CPI index 1 to 4. The results indicate that, in diabetic patients, the presence of a marked impairment of the oral status, as assessed by the CPI index, is accompanied by an increase in plasma and salivary melatonin. The increase in salivary melatonin excretion may have a periodontal protective role.

Key words: community periodontal index, diabetes, interleukin-2, melatonin, saliva

Introduction

Periodontal disease (PD) is an oral inflammatory process affecting the alveolar bone, gums, and periodontal ligament. The etiopathogeny and pathophysiology of the PD is not clear. The presence of microorganisms in the oral cavity initiates a series of processes leading to the damage of healthy tissues. The damage of periodontal tissues results from a direct effect of the toxic products released by the bacteria, and from the action of the immune system stimulated by the bacterial infection [1]. Nevertheless, an important feature in PD is the generation of free radicals, some of which derive from the bacteria themselves, and others originate from the immune response [2]. It is suggested that an increase in both reactive oxygen and nitrogen species during PD is responsible for the oxidative damage to periodontal tissues [3]. The increase in free radical production coexists with a decrease in the antioxidant defense. The imbalance between the prooxidant and antioxidant systems may lead to a further oxidative attack and substantial deterioration of the periodontal tissues [4, 5].

Melatonin is a noteworthy free radical scavenger and a broad-spectrum antioxidant [6–9]. In certain pathologies associated with oxidative stress, melatonin displays both anti-inflammatory and bone repair effects [10–13]. About 24–33% of the plasma melatonin appears in the saliva, where it is easily measured by radioimmunoassay (RIA) [14, 15]. Salivary melatonin measurement provides a readily accessible means of obtaining data on the melatonin excretion via this route. A significant positive correlation between salivary and plasma melatonin exists and the former is a reliable predictor of plasma melatonin levels. By measuring salivary melatonin, oral pathologies can be studied in relation to plasma and salivary melatonin behavior.

In response to antigenic stimuli, T lymphocytes produce interleukin-2 (IL-2). IL-2 regulates a series of processes in different cells of the immune system including natural killer cells, monocytes/macrophages and B lymphocytes. A relationship between IL-2 and melatonin was described when it was found that melatonin stimulates the production of IL-2 by T lymphocytes [16]. It is known that the metabolic products of periodontopathic bacteria decrease cytokine production including IL-2 [17, 18]. Thus, it was of interest
to study the changes in melatonin, IL-2 relationships during periodontal pathologies.

Accordingly, the objective of this study was to assess plasma IL-2 levels and correlate them with plasma and salivary melatonin levels in patients with type I, (DM-I), and type II, (DM-II), with different degrees of PD according to the Community Periodontal Index (CPI) [19]. These data were compared with those measured in a control group of healthy subjects to assess any change in melatonin levels that may be related to the periodontal status and/or immune response.

Materials and methods

Patients

The study was carried out in the Faculty of Odontology (Granada, Spain). A total of 63 patients were included in the study. Information was given and authorization obtained from the patients and from the University's Ethical Committee, and the Code of Ethics of the World Medical Association was observed. Subjects were classified in two groups: a control group that comprised 20 healthy subjects (12 women and eight men), aged 48.9 ± 10.3 yr. The subjects of this group were age- and weight-matched with patients in the diabetic individuals, comprised 43 patients (20 with DM-I and 23 with DM-II). Dental and medical history of all patients was in accordance with the criteria of the WHO. The periodontal status was evaluated by the CPI.

The inclusion criteria for DM patients were: (a) age between 18 and 65 yr old, and (b) glycosylated hemoglobin between 7.6 and 8.0% during the last 6 months, values compatible with a tolerable control of diabetes. Exclusion criteria included the presence of other concomitant systemic pathologies such as epilepsy and schizophrenia, and diseases that may affect the immune system such as chronic infectious and oncological diseases [20, 21]. Patients under pharmacologic treatment that could alter melatonin levels were also excluded from the study. The clinical history also included a dental history following the simplified WHO criteria [19], including both dental (presence or absence of caries) and periodontal (CPI index) status. The oral cavity explorations and clinical data were always analyzed by the same person. A concordant diagnostic analysis was performed, in which other odontologists carried out the oral exploration in 11 cases. This inter-observer assay yielded a concordance coefficient of 81% for the CPI index.

The CPI index is currently recommended by WHO, and it consists of dividing the oral cavity in to six sextant with index teeth in each one. Teeth index are 1/7/16 for the first sextant, 11 for the second, 26/27 for the third, 36/37 for the fourth, 31 for the fifth, and 47/46 for the sixth. Teeth are examined with a probe with two marks at 8.5 and 11.5 mm, which permits the measurement of the dental bone affection’s degree by the inflammatory process. The CPI codes used for recording the periodontal status are the following: code 0, healthy periodont; code 1, moderate bleeding; code 2, presence of supra- or sub-gingival dental calculus; code 3, periodontal sac of 4–5 mm; and code 4, periodontal sac of 6 mm or higher.

Plasma melatonin determination

Patients came to the laboratory at 08:00 hr, and they were seated 30 min before they were sampled. Blood samples were taken from the antecubital vein and centrifuged at 3000 g for 10 min, and plasma was separated and frozen at −20°C until further use. Plasma melatonin was determined by a commercial RIA (DVD BIOCHEMIE, Marburg GmbH, Germany) and a quality control was performed showing an intra- and inter-assay coefficients of variation of 11.3 and 6.3%, respectively. The recovery of added melatonin was 84.4% and the sensitivity of the assay was 4.65 pg/mL.

Salivary melatonin determination

Saliva was obtained after chewing a piece of paraffin. Saliva produced during the first 2 min was discarded. Then, saliva was collected during the following 5 min, avoiding any possible contamination. The patients chewed the paraffin during the time of saliva collection [22]. Samples of collected saliva were centrifuged at 3000 g for 20 min, and the clear supernatant was frozen to −20°C. Melatonin levels in saliva were measured by RIA (IBL, Hamburg GmbH, Germany) [23]. The quality control of melatonin RIA showed an intra- and inter-assay coefficients of variation of 12.9 and 7.2%, respectively. The recovery of added melatonin was 80.3% and the sensitivity of the assay was 2.19 pg/mL.

Plasma IL-2 determination

Plasma IL-2 was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Chemicon International, Temecula, CA, USA). The sensitivity of the method was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 S.D.s read in dose from the standard curve is the lower limit of detection. This value is the smallest dose that is not zero with 95% confidence. The standards in the ELISA were calibrated to the National Institute for Biological Standards and Control (NIBSC) reference lot 86/504. One picogram of endogenous standard = 0.77 NIBSC units. The intra- and inter-assay coefficients of variation were < 10% in each case.

Statistical analyses

Quantitative variables are expressed as the mean ± S.D., whereas absolute and relative frequencies were calculated for qualitative variables. To correlate quantitative with qualitative variables, Mann–Whitney test was used. Student’s t-test was used to compare the mean values of quantitative variables, and the Spearman’s correlation coefficient was used to correlate quantitative variables with qualitative ones. The relationship between one qualitative variable with more than two modalities (CPI index) and the quantitative variables was examined by the Kruskal–Wallis test an/or one-way ANOVA.

Results

Table 1 shows the data corresponding to the control and diabetic subjects. Diabetic patients had significant lower
levels of plasma and salivary melatonin than the corresponding healthy subjects \((P < 0.001)\). Interestingly, the salivary/plasma melatonin ratio was similar in both groups. A significant negative correlation was found between plasma melatonin and age in the control group \((r = -0.681, P < 0.001)\). Interestingly, this correlation disappeared in the diabetic group \((r = 0.02, NS)\).

Table 2 shows the CPI index according to the age of the diabetic patients. Application of Kruskal–Wallis test displayed a \(\chi^2 = 17.78\) \((P < 0.001)\), reflecting the age-dependent increase of CPI index.

We next asked if the diabetes type, DM-I and DM-II, might influence the studied variables. For this purpose, plasma and salivary melatonin and plasma IL-2 levels were studied in relation to the type of diabetes. Fig. 1 shows that, although patients with type II diabetes had higher plasma and saliva melatonin levels than those with diabetes type I, these changes were not significant. IL-2 was also unchanged by the type of diabetes (Fig. 1).

A biphasic relation between plasma melatonin and CPI index was found (Fig. 2). Plasma melatonin decreased in patients with CPI index of 1 (youngest patients) to CPI 2, and then increased reaching highest values at CPI 4. Salivary melatonin parallels the changes of plasma melatonin. IL-2 behaved differently than melatonin (Fig. 2). Highest concentrations of IL-2 were found in diabetic patients with the lowest CPI index. When the CPI index increased, IL-2 decreased, reaching the lowest levels at CPI index of 3. It is of interest to note that IL-2 seems to increase at CPI index of 4, although these changes were not significant.

**Table 1.** Comparison between the studied variables in controls and diabetic patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 20)</th>
<th>Diabetics (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>48.9 ± 10.3</td>
<td>53.8 ± 12.4</td>
</tr>
<tr>
<td>PM</td>
<td>14.91 ± 4.75</td>
<td>8.98 ± 7.14**</td>
</tr>
<tr>
<td>SM</td>
<td>4.35 ± 0.98</td>
<td>2.70 ± 2.04**</td>
</tr>
<tr>
<td>SM/PM</td>
<td>0.30 ± 0.06</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>IL-2</td>
<td>4.00 ± 0.99</td>
<td>4.10 ± 2.07</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. Age is expressed in years and melatonin and IL-2 in pg/mL. n, number of cases. **\(P < 0.001\) versus control.

PM, plasma melatonin; SM, saliva melatonin.

**Table 2.** Mean age of diabetic patients according to the CPI index

<table>
<thead>
<tr>
<th>CPI index</th>
<th>n</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>41.6 ± 11.5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>51.9 ± 9.5</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>58.3 ± 11.2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>63.3 ± 8.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± S.D. n, number of cases.

Fig. 1. Plasma and saliva melatonin and IL-2 levels in relation to the diabetes type. Although plasma and saliva melatonin and IL-2 levels were higher in DM-II patients, only the former showed significant changes \((P < 0.005)\).

Fig. 2. Plasma and saliva melatonin and IL-2 levels in diabetic patients in relation to the CPI index. A transient decrease in plasma and saliva melatonin levels was found at a CPI index of 2. IL-2 decreased significantly from a CPI index 1 to 3 \((P < 0.05)\).
Discussion

The current results show that either in control group or in diabetic patients, melatonin excretion in saliva corresponds to 30–31% of plasma melatonin, which is in agreement with similar values previously reported in humans [24, 25]. The percentage of melatonin extraction in saliva is independent of the plasma melatonin concentration. However, the absolute concentration of salivary melatonin changes as a function of its plasma levels. Consequently, inflammatory processes such as periodontitis, which trigger signals to increase plasma melatonin concentration, also increase melatonin levels in the oral cavity, where the indoleamine may exert a protective role. Salivary melatonin determinations seem valid for studies on melatonin behavior in oral pathologies, and yield reliable data with a non-invasive procedure, which is very useful for the odontologist [26, 27].

According to the CPI index, the periodontal status deteriorates with age. Melatonin levels significantly decreased from CPI index 1 to 2, which may be related to the age-dependent reduction in both melatonin production and salivary levels [28, 29]. An interesting finding in this study is that, when the CPI index is 3–4, corresponding to a serious periodontal process with bone damage and gingival involvement, melatonin levels increase. Thus, patients with higher CPI indexes, and thus older than those with lower CPI index, have high melatonin levels. Since higher CPI indexes correspond to a worst periodontal status, the melatonin increase may be a consequence of a signal(s) derived from the oral inflammatory process [30, 31]. It is suggested that the organism responds to a periodontal inflammation by increasing melatonin production and thus, melatonin availability to the oral cavity. Melatonin may participate in the restoration of alveolar bone stimulating the proliferation of type I collagen cells [32] and modulating both osteoclastic and osteoblastic activities [12, 13, 33, 34].

It was shown that lymphocytes of patients with DM-I produce low IL-2 [35]. However, no correlation between IL-2 and periodontal status was investigated in these reports. Our results show that IL-2 levels are unrelated to the type of diabetes but they significantly change with the periodontal status reaching lowest levels at highest CPI indices. Perhaps the significant melatonin increase at CPI 4 may be somewhat responsible for the slight elevation in IL-2 [16, 36]. Patients with DM-I show a lesser response in both melatonin and IL-2, suggesting that the immunostimulating and anti-inflammatory properties of melatonin are also depressed in these patients. It is important to note that in these patients, periodontal processes yield premature involvement of both alveolar and dental bone [10, 11, 37–39].

Previous data have shown the existence of an inverse relationship between peroxidation products and the quantity of antioxidants in periodontal pathology [40]. A key finding in periodontitis is polymorphonuclear neutrophils infiltration; these cells produce high amounts of reactive oxygen species (ROS). Moreover, a massive neutrophil migration to the gingiva and gingival fluid during periodontitis leads to abnormal spreading of ROS [41]. At least part of the antioxidant potential in the oral cavity relates to uric acid and, to a lesser extent, to vitamin C and albumin [42, 43]. No clear evidence has emerged in relation to the possible antioxidant activity of vitamin A or CoQ in periodontitis. Some studies show that a deficiency in vitamin E, another antioxidant, does not increase in periodontitis; in general, studies do not provide any support for the treatment of periodontitis with vitamin E. An interesting observation is that in older subjects more affected by periodontitis, vitamin E tended to increase [41]. Our results show a significant increase in melatonin levels in older patients having more periodontal damage. Thus, the melatonin rise in PD may also be secondary to the increase in the free radical production in this pathology. Due to the antioxidant [6–9, 44, 45] and anti-inflammatory [46] effects of melatonin, the increase in salivary melatonin levels may improve the organism’s response against the periodontal inflammatory process. These data agree with a role of melatonin against diabetes-induced oxidative stress [47, 48] and suggest that melatonin may participate in the anti-oxidative defense against free radical attack into the oral cavity.

From the above data, at least three important actions of melatonin, i.e. immunoenhancing, fibroblast proliferation and bone remodeling, and antioxidant, may be related to the presence of melatonin into the mouth. The importance of melatonin as an antioxidant in the oral cavity depends on its parallel effect on the immune system, which differs from other antioxidants such as vitamins A, E and CoQ, although the latter has a minor immunostimulatory role [49]. Also, it has been repeatedly shown that melatonin exerts a protective role against free radical damage during diabetes [50]. Thus, a melatonin increase during diabetes could protect all organs from oxidative damage, but this may be of particular importance into the mouth. Further studies on the melatonin effects into the oral cavity including its oxidative metabolites, would help to clarify the role(s) of melatonin into the mouth and the potential of its use in oral hygiene.

Acknowledgments

This work was partially done with grants FIS 01/1076 from the Instituto de Salud Carlos III, Spain, and CTS-263 and CTS-101 from the Consejerı¨a de Educacio ´n, Junta de Andalucı´a, Spain.

References


