Serum and Saliva Sirtuin 6, Lipoxin A4, Caspase8 Levels in Correlation with Periodontal Status in Severe Periodontitis

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Research Article

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Abstract

Objectives: It has been stated that Siruvin-6 (SIRT6) play a important role in regulation of inflammation, energy metabolism, homeostasis and apoptosis, and SIRT6 may be assosiciated with many diseases. The aim of this study was to evaluate saliva and serum SIRT6, Lipoxin A4 (LXA4) and Caspase-8 (CASP8) levels in correlation with periodontal clinical status in patients with periodontitis and healthy subjects.

Materials and Methods: 20 patients with Stage III Grade B periodontitis (P) and 20 periodontally healthy individuals (control;C) were included in this study. Clinical periodontal parameters were measured. Saliva and serum levels of SIRT6, LXA4 and CASP8 were analyzed by enzyme-linked immunosorbent assay.

Results: Serum SIRT6 and saliva LXA4 levels were significantly lower in the periodontitis group than in the control group (respectively; p=0.010, p= p:0.001). There was no statistically significant difference between the periodontitis and control groups for saliva SIRT6, serum LXA4, serum and saliva CASP8 levels (p>0.05). Significant negative correlations were found between all periodontal clinical parameters (PI, BOP, PPD, CAL) and saliva LXA4 level (p<0.05), and between PPD, CAL, and serum SIRT6 level (respectively; r=-0.465 and r=-0.473, p<0.05).

Conclusions: This study demonstrated that significantly lower levels of serum SIRT6 and saliva LXA4 in periodontitis patients and their correlation with periodontal status. Detection of SIRT6 and saliva LXA4 might have a potential role for predicting periodontal status in the future with more precise sampling protocols and with better specity in testing methods.

Clinical Relevance: Serum SIRT6 and saliva LXA4 might be promising biomarkers for monitoring the susceptibility to periodontitis and predicting periodontal status.

Introduction

Inflammation is a protective response against infection or injury to maintain homeostasis. Inflammatory lesions are generally hypoxic and most of the cellular responses to hypoxia are mediated by the production of reactive oxygen species (ROS) [1]. Hypoxia affects osteogenic differentiation, oxidative stress and apoptotic pathways [2].

Apoptosis is a major cellular event involved in development of homeostasis and has a significant role in the regulation of the host immune response and inflammation [3]. Apoptosis is initiated by extrinsic and intrinsic signaling pathways. Caspase 8 (CASP8) is a member of cysteine proteases that triggers apoptosis with cleavage of downstream effector caspase-3 via extrinsic signaling pathways [4]. CASP8 has been associated with neurogenerative conditions such as Parkinson's and Alzheimer's diseases [5]. The inhibition of CASP8 also have been proposed as potential treatments for these conditions [6].
In the intrinsic signaling pathways, BAX leading to activate caspase-9 which in turn cleaves caspase-3, causing in the downstream events related in apoptosis. The release of BAX is regulated by the Ku70 that is DNA repair factor. Sirtuin 6 (SIRT6) inhibits stress-mediated apoptotic cell death by deacetylating Ku70 [7]. SIRT6 is one of seven members of the family of NAD + dependent protein deacetylases. It is a crucial regulator of apoptosis and able to preserve hypoxic damage by decreasing ROS generation [8]. SIRT6 plays a significant role in inflammatory pathways and has anti-inflammatory effects [9, 10]. Several studies reported that SIRT6 deficiency is associated with the onset of multiple pathologies, including metabolic syndrome, diabetes, cardiovascular disease, neurodegenerative disease, cancer and that SIRT6 may be a potential therapeutic target [11, 12]. Lipoxin A4 (LXA4) is known pro-resolving lipid mediator of inflammation and inhibits hypoxia-induced apoptosis and oxidative stress [13]. It has been reported that LXA4 reduced caspase 3, -8 and – 9 activation [14]. Several studies suggested that decreased LXA4 levels are related with many inflammatory disorders such as diabetes, coronary heart diseases, metabolic syndrome, rheumatoid arthritis, and that LX replacement therapy may offer a new therapeutic approach [15, 16].

Periodontitis is a chronic inflammatory disease characterized by destruction of tooth-supporting structures as a result of the immune-inflammatory response of the host against pathogenic bacteria in dental biofilm [17]. Also, it has been shown that apoptosis is one of the mechanisms involved in the pathogenesis of periodontitis [18]. There are some studies those have reported that LXA4 is associated with periodontitis, and its metabolically stable analogs resolves this inflammation [19, 20]. A recent study reported that the extrinsic pathway involving CASP8 plays a role in aggressive periodontitis [21]. However, different findings have been presented concerning the role of CASP8 in chronic periodontitis [21, 22]. Several studies reported that the development of apical periodontitis is correlated by decreased expression of SIRT6 and that SIRT6 may reduce periapical lesions by inhibiting apoptosis [23, 24]. A recent article revealed that SIRT6 inhibits the inflammatory response of LPS-induced periodontal ligament stem cells (PDLSCs) by inhibiting the nuclear factor κB (NF-κB) pathway, while promoting viability and osteogenic differentiation [25]. The apoptosis mechanism is triggered in the hypoxic environment that occurs as a result of the prolongation of the inflammatory response in the periodontal tissues (chronic inflammation). It is thought that there are additional molecules that stop or slow down this mechanism such as Lipoxin A4 which plays an active role in the resolution of inflammation, and Sirtuin 6 which was previously found to inhibit apoptosis in endodontic apical lesions [20, 24]. To the best of our knowledge, no study has assessed the possible role SIRT6 and its relationship with LXA4 in periodontitis patients. We hypothesized that Sirtuin 6 may play a role in regulating hypoxia-induced apoptosis and resolution of inflammation in periodontitis. Thus, the aim of this study was to evaluate saliva and serum LXA4, CASP8 and SIRT6 levels in correlation with periodontal clinical status in patients with periodontitis and healthy subjects.

Materials And Methods

Study population
The study protocol (Date: 03.02.2022, no: 138) was approved by the Ethical Committee of Medipol University, Istanbul, Turkey in accordance with Helsinki Declaration 1975, as revised in year 2000. This study involved a total of 40 subjects, comprising systematically healthy control group with a healthy periodontium (C group; 11 females and 9 males; mean age: 32 ± 7.43 years) and systematically healthy periodontitis group with Stage III Grade B generalized periodontal disease (P group; 10 females and 10 males; mean age: 39.7 ± 7.56 years). All participants were recruited from the clinics of Istanbul Medipol University and Istanbul University Faculty of Dentistry Department of Periodontology. They were given oral and written information about the study protocol and their informed consent was obtained.

The exclusion criteria were as follows: being a smoker, using antibiotics and/or anti-inflammatory nonsteroidal anti-inflammatory drugs, steroids, immunosuppressants, beta-blockers, calcium channel blockers, anticoagulants, and hormonal contraceptives within 3 months preceding the study; having nonsurgical periodontal treatment within 6 months; having surgical periodontal treatment within 12 months; having less than 20 natural teeth excluding the third molars; and having a diagnosis of diabetes, rheumatoid arthritis diagnosis, or systemic conditions including human immunodeficiency virus infection and acquired immunodeficiency syndrome, cardiovascular disorders, epilepsy, renal disorders, or hepatic disorders, or experiencing pregnancy, lactation.

Clinical periodontal examination and diagnosis

A full-mouth periodontal examination included the plaque index (PI), probing pocket depth (PPD), gingival recession (GR), clinical attachment level (CAL), and bleeding on probing (BOP) was performed by a single calibrated examiner (SS). All measurements were recorded at 4 sites (mesio-buccal, mid-buccal, disto-buccal, lingual/palatinal) per tooth by using a manual periodontal probe (William's probe, Hu-Friedy, Chicago, IL). Average scores for whole mouth for PPD, CAL, GR in mm, and the percentage of sites with BOP were calculated for each subject. After periodontal examinations, healthy periodontium was defined as no sites with a probing pocket depth (PPD) >3 mm, as well as no signs of inflammation (BOP <10%) whereas patients who had interproximal attachment loss of ≥2 mm in at least one tooth were diagnosed with periodontitis (26). For each tooth, a call of most severe sites were recorded and Stage III was defined as to have a call of CAL ≥5 mm. In addition, periodontitis patients were graded according to the bone loss/age index as Grade B (index score between 0.25 and 1.00) according to the 2017 World Workshop for classification of periodontal and peri-implant diseases [26].

Saliva and serum sampling

Saliva were collected to analyze the selected markers as unstimulated samples during the early hours of the day. The patients were asked to rinse their mouth with distilled water and then spit into plastic tubes for 10 minutes. The saliva was centrifuged for 10 min at 2800 g and then transferred into Eppendorf tubes [27].

Venous puncture was performed after saliva collection and 10 mL of blood samples were collected by qualified staff (MFD) from each participant. To separate the serum, samples centrifuged at 4000 g for 10 minutes. Saliva and serum were then stored at −80°C until analysis [28].
Saliva and serum samples processing and analyses

Saliva and serum samples obtained for each patient were used for cytokine analysis. Prepared samples were analyzed for Lipoxin A4 (LXA4), Caspase 8 (CASP8) and Sirtuin-6 (SIRT6) using commercial ELISA kits (Elabscience, Houston, Texas, USA and Bioaasay Technology Laboratory (BT-Lab), Shanghai, China, respectively) according to the manufacturer's instructions. The detection limits of ELISA kits were 0.78–50 ng/ml for LXA4, 0.16–10 ng/ml for CASP8 and 0.1–40 ng/ml for SIRT6.

Statistical analysis

We used IBM SPSS Statistics program version 22.0 (SPSS v.22, IBM SPSS Inc., Chicago, IL, USA) to analyze the data. The power analysis was performed using a specific software (3.1.9.2 G*Power; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html) before the initiation of the study. Using a large effect size (1.1) with a 0.05 α-error, and 80% power, the resulting total sample size was 28. However, considering the possibility of confounders and incomplete data, the study was designed to include 40 patients [28].

Kolmogorov-Smirnov and Shapiro-Wilk test was applied to determine data normality. In brief, where there was direct concordance in sample numbers between disease categories, the Wilcoxon test was used for intragroup comparisons of dependent samples. For intergroup comparisons, where the parameters were distributed normally, the Student's t test was used to compare the differences between the parameters recorded while the Mann-Whitney U test was used for not normal distributed parameters. Fisher Freeman Halton Exact Chi-square test and Continuity (Yates) Correction were used to compare qualitative data. Intergroup comparisons of biochemical and oral hygiene habits data were assessed using the Kruskal-Wallis test. To determine how saliva and serum LXA4, CASP8 and SIRT6 levels were related to clinical periodontal parameters, Spearman correlation test was used. All tests were performed at a significance level of α = 0.05.

Results

Demographic and clinical parameters

Demographic and full mouth clinical periodontal parameters are reported in Table 1. The mean age and BMI and, all clinical periodontal measurements (PI, PPD, GR, CAL, BOP) were found to be significantly higher in the periodontitis group than the controls (p < 0.05). There was no statistically significant difference between the groups in terms of gender distribution (p > 0.05)
Table 1
Demographic, clinical and biochemical results of periodontitis and control groups

<table>
<thead>
<tr>
<th></th>
<th>Control (C) n = 20</th>
<th>Periodontitis (P) n = 20</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)*</td>
<td>32 ± 7,43</td>
<td>39,7 ± 7,56</td>
<td>0,002</td>
</tr>
<tr>
<td>Gender F/M</td>
<td>11 / 9</td>
<td>10 / 10</td>
<td>1,000</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>22,63 ± 2,42</td>
<td>27,05 ± 4,5</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Plaque Index**</td>
<td>0,35 ± 0,45</td>
<td>1,58 ± 0,46</td>
<td>0,001</td>
</tr>
<tr>
<td>Pocket probing depth (mm)**</td>
<td>1,62 ± 0,23</td>
<td>3,62 ± 0,99</td>
<td>0,001</td>
</tr>
<tr>
<td>Bleeding on probing (%)**</td>
<td>7 ± 2,51</td>
<td>47,11 ± 24,45</td>
<td>0,001</td>
</tr>
<tr>
<td>Clinical attachment loss (mm)**</td>
<td>0,27 ± 0,84</td>
<td>4,14 ± 1,4</td>
<td>0,001</td>
</tr>
<tr>
<td>Saliva SIRT6 (ng/mL)</td>
<td>14,3 ± 4,58</td>
<td>16,32 ± 3,21</td>
<td>0,119</td>
</tr>
<tr>
<td>Saliva CASP8 (ng/mL)</td>
<td>0,16 ± 0,23</td>
<td>0,15 ± 0,1</td>
<td>0,877</td>
</tr>
<tr>
<td>Saliva LXA4 (ng/mL)**</td>
<td>1,28 ± 0,71</td>
<td>0,69 ± 0,32</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Serum SIRT6 (ng/mL)*</td>
<td>15,37 ± 5,05</td>
<td>10,53 ± 4,62</td>
<td>0,010</td>
</tr>
<tr>
<td>Serum CASP8 (ng/mL)</td>
<td>0,07 ± 0,05</td>
<td>0,06 ± 0,03</td>
<td>0,849</td>
</tr>
<tr>
<td>Serum LXA4 (ng/mL)</td>
<td>26,1 ± 31,19</td>
<td>26,56 ± 39,79</td>
<td>0,344</td>
</tr>
</tbody>
</table>

C, group of periodontally and systemically healthy volunteers; P, group of systemically healthy patients with Stage III Grade B generalized periodontitis.

Data are expressed as mean ± SD

* Statistically significant difference between test and control groups (p < 0.05; Student t test)

** Statistically significant difference between test and control groups (p < 0.05; Mann–Whitney U-test).

Biochemical parameters

The biochemical findings of periodontitis and control groups are presented in Fig. 1 and Table 1. Serum SIRT6 and saliva LXA4 levels were significantly lower in the periodontitis group compared with the controls (respectively; p = 0.010, p = p:0.001).

There was no statistically significant difference between the groups in terms of saliva SIRT6, serum and saliva CASP8, and serum LXA4 levels (p > 0.05).

Correlation analysis of clinical and biochemical parameters
The correlations between the clinical and biochemical parameters are reported in Table 2. Serum SIRT6 was negatively correlated with PPD and CAL (respectively; r=-0.465 and r=-0.473, p < 0.05), whereas saliva SIRT6 was positively correlated with these (respectively; r = 0.326 and r = 0.344, p < 0.05). Saliva LXA4 was negatively correlated with all clinical parameters, and was most strongly correlated with PPD (r=-0.539, p < 0.001), whereas serum LXA4 and serum and saliva CASP8 and were not significantly correlated with any clinical parameters. (p > 0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Serum SIRT6</th>
<th>Saliva SIRT6</th>
<th>Serum CASP8</th>
<th>Saliva CASP8</th>
<th>Serum LXA4</th>
<th>Saliva LXA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>r -0.225</td>
<td>0.238</td>
<td>0.152</td>
<td>0.224</td>
<td>-0.029</td>
<td>-0.465</td>
</tr>
<tr>
<td></td>
<td>p 0.224</td>
<td>0.144</td>
<td>0.459</td>
<td>0.317</td>
<td>0.858</td>
<td>0.003*</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>r -0.465</td>
<td>0.326</td>
<td>0.012</td>
<td>0.087</td>
<td>-0.18</td>
<td>-0.539</td>
</tr>
<tr>
<td></td>
<td>p 0.008*</td>
<td>0.043*</td>
<td>0.955</td>
<td>0.7</td>
<td>0.266</td>
<td>0.000*</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>r -0.378</td>
<td>0.288</td>
<td>0.134</td>
<td>0.088</td>
<td>-0.156</td>
<td>-0.418</td>
</tr>
<tr>
<td></td>
<td>p 0.036</td>
<td>0.076</td>
<td>0.514</td>
<td>0.696</td>
<td>0.335</td>
<td>0.007*</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>r -0.473</td>
<td>0.344</td>
<td>0.309</td>
<td>-0.082</td>
<td>-0.126</td>
<td>-0.457</td>
</tr>
<tr>
<td></td>
<td>p 0.007*</td>
<td>0.032*</td>
<td>0.125</td>
<td>0.716</td>
<td>0.44</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*p < 0.05

The correlations between serum and salivary levels of SIRT6, CASP8, and LXA4 are presented in Table 3. Serum SIRT6 was negatively correlated with saliva SIRT6 (r=-0.622, p < 0.001). Also, there was a negative correlation between saliva LXA4 and saliva CASP8 (r=-0.654, p < 0.05). There was no statistically significant correlations between other biochemical parameters (p > 0.05).
Table 3
Correlations between serum and salivary levels of SIRT6, CASP8, and LXA4 (Spearman and Pearson's correlation coefficients, r)

<table>
<thead>
<tr>
<th>Biochemicals</th>
<th>a Serum SIRT6</th>
<th>a Saliva SIRT6</th>
<th>Serum CASP8</th>
<th>Saliva CASP8</th>
<th>Serum LXA4</th>
<th>Saliva LXA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum SIRT6</td>
<td>r 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva SIRT6</td>
<td>r -0.622</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p 0.000*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum CASP8</td>
<td>r 0.094</td>
<td>-0.008</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saliva CASP8</td>
<td>r 0.459</td>
<td>-0.13</td>
<td>0.094</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p 0.064</td>
<td>0.564</td>
<td>0.738</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum LXA4</td>
<td>r -0.233</td>
<td>0.245</td>
<td>-0.337</td>
<td>-0.028</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Saliva LXA4</td>
<td>r 0.222</td>
<td>0.027</td>
<td>0.032</td>
<td>-0.654</td>
<td>0.191</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>p 0.23</td>
<td>0.872</td>
<td>0.877</td>
<td>0.001*</td>
<td>0.238</td>
<td></td>
</tr>
</tbody>
</table>

a Pearson correlation analysis was used for all correlations with serum and salivary SIRT6. *p < 0.05
Spearman's rho correlation test was used for correlations between other parameters. *p < 0.05

Discussion

Periodontitis is a chronic inflammatory disease that involves hypoxia-induced apoptosis mechanisms [18]. Caspase activation has been reported to be associated with periodontitis through this mechanism, and few studies have investigated CASP8 in periodontitis [21, 22]. Also, it has been reported that SIRT6 modulates hypoxia-induced apoptosis in many studies, and this has been shown in endodontic periapical lesions [23, 24]. However, there is no study assessed the possible role SIRT6 in pathogenesis of periodontitis. In addition, LXA4, which has been associated with periodontitis through inflammatory pathways in various studies, has recently been shown to play a role in the mechanism of hypoxia-induced apoptosis. Therefore, we evaluated serum and saliva levels of SIRT6, CASP8 and LXA4 in patients with stage III/grade B periodontitis.

In this study, it was demonstrated that serum SIRT6 and saliva LXA4 levels were significantly lower in stage III/grade B periodontitis patients than healthy controls. We have shown decreased serum levels of SIRT6 were associated with advanced periodontitis for the first time. SIRT6 is known to be involved in the
regulation of inflammation, stress, energy metabolism, and cell survival [29]. SIRT6 inhibits stress-mediated apoptotic cell death by cleaving BAX from mitochondria [7]. It has been shown that it is a critical regulator of glucose homeostasis and apoptosis, and protects the heart from hypoxic damage by reducing ROS production [8]. Balestrieri et al. (2015), reported that SIRT6 expression in atherosclerotic lesions of Type 2 diabetes (T2DM) patients was downregulated, and the decreased SIRT6 expression was related with increased oxidative stress and inflammation [30].

SIRT6 has anti-inflammatory effects by inhibiting the expression of NF-κB target genes and other pro-inflammatory cytokines [9]. It has been reported that overexpression of SIRT6 inhibits inflammatory responses and bone destruction in mice with collagen-induced arthritis [31]. Also, Woo et al. (2018), demonstrated that myeloid SIRT6 deficiency augments rheumatoid arthritis by enhancing macrophage activation and infiltration [32]. Other studies have associated SIRT6 deficiency with increased osteoclastic bone resorption and osteopenia [33, 34]. Hou et al. (2016), demonstrated that SIRT6 supresses hypoxia-induced inflammatory response in human osteoblasts by inhibition of ROS generation and glycolysis [35]. Periodontitis is characterized by increased inflammation, oxidative stress and bone destruction, and findings of these studies reported in SIRT6 deficiency are also involved in the pathogenesis of periodontitis and are consistent with our study.

Also, a study by Kok et al. (2015) revealed that SIRT6 modulates hypoxia-induced apoptosis in osteoblasts by inhibition of glycolysis and, it might alleviate periapical lesions [23]. Another study reported that SIRT6 supresses periapical lesion progression by modulating hypoxia-induced chemokine ligand 2 production in osteoblasts [24]. These findings may explain the low serum SIRT6 level in periodontitis, which is probably associated by hypoxia-induced apoptosis according to the previous studies.

Additionally, SIRT6 deficiency was associated with activation of NF-κB pathway in LPS-induced human dental pulp cell [36]. A recent study by Li et al. (2022), demonstrated that SIRT6 supresses the inflammatory response of LPS-induced PDLCs via inhibiting NF-κB pathway and, it promotes osteogenic differentiation and viability [25]. PDLCs represent local immune cells of the periodontal tissues and SIRT6 may have possible role of development periodontitis via NF-κB pathway. However, in the present study, we did not find a significant difference in salivary levels of SIRT6 between patients with periodontitis and healthy individuals. While SIRT6 in many diseases are mostly investigated in the serum [37–39], saliva SIRT6 level has been analyzed in only one study [40]. SIRT6 level fluctuation in saliva may not be detected easily because of the complex structure and content of saliva [41]. Thus, we think that serum level of SIRT6 is more important in monitoring the possible role of SIRT6 in periodontitis.

In the current study, we have also shown that LXA4 level in saliva decreased with periodontitis. Several studies have reported that LXA4 is related with periodontitis and its replacement therapy resolves periodontal inflammation in animal models [19, 20]. Consistent with our findings, a recent study by Tobon-Arroyave et al. (2019), reported that lower salivary LXA4 level was detected in periodontitis patients compared to healthy subjects [42]. However, we found no difference in serum levels of LXA4 in
periodontitis than healthy individuals. Contrary to this study, few studies have reported increased serum LXA4 levels in chronic and aggressive periodontitis [43, 44].

In previous studies using GCF samples LXA4 level was reported to be lower in periodontitis, confirming that the findings of decrease in salivary LXA4 might be an indicator of susceptibility to periodontitis [45–47]. Therefore, saliva level of LXA4 may be more reliable than serum in evaluating the its role in periodontitis. LXA4 is a pro-resolving and anti-inflammatory mediator that acts inhibiting leukocyte-dependent inflammation. Similar to SIRT6, LXA4 inhibits the activation of NF-κB and this effect decreased the production of the inflammatory cytokines [48]. Liu et al. (2022), demonstrated that combination of Resolvin E1 and LXA4 treatment in pulpitis downregulated NF-κB activation and increased the expression of SIRT6 [49]. Our results might indicate that SIRT6 is involved in LXA4-mediated resolution and might synergistically promote resolution of inflammation in periodontitis. Also, it has been reported that LXA4 inhibits hypoxia-induced apoptosis and oxidative stress in human first trimester trophoblast cells [13]. Additionally, it has been shown that LXA4 reduced caspase 3, -8 and −9 activation [14]. The role of LXA4 in periodontitis might also be related to apoptotic mechanisms.

It is thought that caspase activation might have an important role in periodontitis-associated tissue damage. Recently, several studies revealed that increased levels of caspase-3 is associated with periodontal disease progression [50–52]. We have evaluated saliva and serum CASP8 levels and found no significant differences between periodontitis patients and healthy individuals. Aral et al. (2017), reported that GCF levels of CASP8 similar between individuals with chronic periodontitis and healthy controls [21]. Also, Shi et al. (2019), evaluated CASP8 in gingival tissues and they found no differences between chronic periodontitis patients and healthy subjects [53]. These results are consistent with our study. Aral et al, (2017), also found that decreased saliva levels of CASP8 in aggressive periodontitis [21]. Manosudprasit et al. (2017), shown that in peripheral blood neutrophils, decreased CASP8 levels in chronic periodontitis with and without T2DM compared to healthy controls [22]. Several studies emphasize that caspase-3 and −8 play an essential role in the butyric acid-induced apoptosis of inflamed gingival fibroblasts [54, 55]. Also, Zhou et al. (2018), reported that Porphyromonas gingivalis LPS induced apoptosis via increases expressions of caspase-3 and −8 in osteoblasts [56]. ROS activate CASP8 to induce apoptosis and increases the BAX/BCL-2 ratio. Caspase-8, an initiator in extrinsic signaling pathways, cleaves caspase-3, an effector caspase, resulting in downstream events involved in apoptosis. Caspase-3 is rapidly activated by CASP8 [57]. The reason that we could not detect differences in CASP8 levels between groups might be due to the short half-life of this molecule and having a short detection window. SIRT6 has been shown to have an important role on caspase-8 in cancer cells by differentially regulating the expression and activity of pro-apoptotic and anti-apoptotic factors, depending on the type and stage of cancer [58]. The relationship of SIRT6 with CASP8 in its potential role in periodontitis can be better explained by using GCF samples as it may reflect the response in specific sites, and also by evaluating it together with effector caspase, caspase-3.

In the current study, negative correlations were found between all clinical parameters and saliva LXA4 level, and between PPD, CAL, and serum SIRT6 level. These findings regarding the correlation between
salivary LXA4 and periodontal status is consistent with a previous study [42]. Our study findings demonstrated that decreased saliva LXA4 and serum SIRT6 levels are associated with periodontitis and indicating that decreased levels of these molecules would help to predict the clinical signs of the periodontal disease. Also we have found negative correlations between the SIRT6 serum and saliva levels, and CASP8 and LXA4 levels in saliva. The lack of previous studies on these correlations and also the fact that we have not seen significant differences between groups for these two molecules in our study, make it difficult to compare and interpret these correlations.

The main limitation of the current study is the relatively limited number of participants. Another limitation is the analysing only CASP8 as an indicator of apoptotic pathways. Also, the lack of a gingivitis group can be considered as a limitation.

In the current study, the results have underlined the importance of SIRT6, LXA4 and their correlation with periodontal status in periodontitis. Lower serum SIRT6 and saliva LXA4 levels in severe periodontitis may suggest SIRT6 and LXA4 are two of the many key molecules that are regulating periodontal health. Nonetheless, further analysis and larger studies are needed to clarify the role of SIRT6 in periodontal pathogenesis and resolution of inflammation processes.

Conclusion

This is the first study evaluating serum and saliva levels of SIRT6 in periodontitis. This study demonstrated that significantly lower levels of serum SIRT6 and saliva LXA4 in periodontitis patients and their correlation with periodontal status. Detection of SIRT6 and saliva LXA4 might have a potential role for predicting periodontal status in the future with more precise sampling protocols and with better specificity in testing methods.

Declarations

ACKNOWLEDGMENTS

Declared none.

AUTHOR CONTRIBUTIONS

AC contributed to the design of the study, recorded clinic data, and wrote the manuscript with input from other authors. SS contributed to the design of the study, collected the samples and helped interpret the results and wrote the manuscript with input from other authors. MFD contributed to the design of the study, collected the samples and helped interpret the results. ET helped to collect the samples, recorded and analysed clinic data, and wrote the manuscript with input from other authors. NB contributed to the design of the study, performed statistical analysis, and helped interpret the results. HT recorded clinic data, and wrote the manuscript with input from other authors. All authors reviewed and approved the submitted manuscript.
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**Conflicts of interest**

The authors declare that they have no conflict of interests.

**Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent**

Informed consent was obtained from all individual participants included in the study and/or their relatives or legal representatives.

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Figure 1

Serum and saliva levels of SIRT6, CASP8 and LXA4 for patients with systemically healthy patients with Stage III Grade B periodontitis (P) and periodontally and systemically healthy volunteers (C). Box-and-whisker plots with the median (horizontal line), interquartile range (box) and outlier (circles) values are shown. *Significantly different (p < 0.05) from the control group. Intergroup differences were determined using the Mann–Whitney U-test and Student t test.