Abstract: The purpose of the present study was to evaluate periodontal health status in children with sickle cell disease (SCD). Forty-nine children with SCD and 39 systemically healthy sex- and age-matched children were enrolled in the study. Plaque index, gingival index, bleeding on probing, pocket depth, salivary volume, and hyperplastic index were recorded. In addition, the histopathological evaluation of gingiva was made in a child with SCD. There were significant differences between the groups with regard to hyperplastic index \((P < 0.05)\), whereas there were no differences in other parameters. Gingival enlargement was detected in 27 children (55.1\%) in the SCD group and 6 children (15.4\%) in the control group \((P < 0.001)\). However, there were no differences in periodontal health status of children in the SCD and control groups. The most important finding of this study that the gingival enlargement was more prevalent in children with SCD. Sickling and chronic inflammation seen in SCD may affect gingival tissues. Therefore, physicians and dentists must be aware of the effects of SCD on gingival tissues.

Keywords: sickle cell disease; gingiva; gingival enlargement; histopathology.

Introduction
Sickle cell disease (SCD) is a fatal hereditary disease in which acute illness attacks and detrimental organ damage are observed. A point mutation in the hemoglobin (Hb) \(\beta\) chain gene leads to SCD. The electrophoretic abnormalities in sickle hemoglobin (HbS) are pathognomonic for SCD (1). The estimated prevalence of SCD in the world is 7%. While the disease is mostly seen in Africa, it has spread over the whole world as a consequence of migration. The major hemoglobinopathy seen in Turkey is HbS, with a prevalence of 0.37-0.6\%, but it is more prevalent in the East Mediterranean region (3-44\%) (2).

The pathogenesis of SCD originates from the polymerization of deoxygenated HbS. This polymer formation alters the normal biconcave disc shape into a rigid, irregularly-shaped, unstable cell (3). Abnormal structured sickle hemoglobin, which results in the sickle shape of erythrocytes, polymerizes within the cell. Sickled erythrocytes create a thin occlusion within vessels, resulting in tissue ischemia (3).

The two important features of SCD are chronic hemolytic anemia and vaso-occlusion, resulting in ischemic tissue injury (1). The essential feature of the disease is “sickle crisis”, resulting in life-threatening complications including acute pain, infection, stroke, acute chest syndrome, growth retardation, organ damage, and osteomyelitis (1).

SCD is a chronic inflammatory disease characterized by elevated leukocyte counts, hyper-reactive granulocytes, monocytes, and endothelial cells, and increased levels of pro-inflammatory mediators (4,5). Chronic inflammation is observed in patients who are in the steady state, whereas elevated acute inflammatory cytokines are one of the important causes of vaso-occlusion in SCD (6).
Some oro-facial changes including mid-facial overgrowth, facial swelling, and osteonecrosis were reported in SCD patients (7-9). Few studies exist regarding the periodontal status of patients with SCD (10-13), but their results are conflicting. In one study, it was reported that patients with SCD have greater periodontal pocket depths than those of their healthy counterparts (10). However, other studies stated that there were no differences between patients with SCD and controls with regard to probing depth or attachment loss (11-13). In addition, higher plaque index (PI), gingival index (GI), and bleeding on probing (11,12) scores in subjects with SCD were reported.

The present study aimed to explore the periodontal health status of 5-18 year-old children with SCD and compare them with those of their healthy counterparts.

**Materials and Methods**

The present study was performed in cooperation with the Department of Pediatric Hematology at Mersin University, Faculty of Medicine and the Department of Periodontology at Süleyman Demirel University, Faculty of Dentistry. The research protocol was independently reviewed and approved by the Mersin University Faculty of Medicine Ethical Committee (date: 10 January 2013; number: 2013/15). The study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki which was revised in 2008. This study was carried out from October 2013 to October 2014.

**Study groups**

The study population consisted of 49 children with SCD (18 girls and 31 boys, age range 5-18) (SCD group) who were followed by the Pediatric Hematology Clinic in Mersin University, Faculty of Medicine, Department of Pediatrics and 39 systemically healthy children (19 girls and 20 boys, age range 5-18) (control group), who had been admitted to Mersin University, Faculty of Medicine, Department of Pediatrics for routine control and the patients subsequently volunteered for the study. Informed written consent was obtained from all the children and their parents.

Individuals using medication affecting gingival status, such as phenytoin derivatives, cyclosporine, or calcium-channel blockers, were excluded from the study. None of the participants had received periodontal therapy and orthodontic treatment or used antibiotics and non-steroidal analgesics in the month prior to the study.

All the children with SCD were physically examined by an experienced pediatrician (S.Ü.). Medical status of the SCD patients was evaluated to identify the subjects meeting the inclusion criteria. The type of SCD (genotypes of the subjects with SCD were confirmed by β-gene mutation analysis), the frequency of blood transfusions, and vaso-occlusive painful crisis in the last year, the history of acute chest syndrome, splenectomy, and stroke and the usage of hydroxyurea, penicillin prophylaxis, chelating agents, and other medication were evaluated and recorded in children with SCD.

Participants with SCD who had taken penicillin prophylaxis (1,200,000 IU Benzathine- Penicillin G/month) within the last 30 days before enrolment were included in the study after completion of 30 days of antibiotic usage (14). In addition, participants with SCD receiving a blood transfusion or having a vaso-occlusive crisis in the previous month were excluded from the study.

The study included children aged 5-18 years with different dentitions (deciduous, mixed, and permanent). Therefore, the SCD and control groups were categorized and sub-grouped according to their dentition status. The study groups formed were as follows: SCD₃, SCD patients with deciduous or mixed dentition; SCDₚ, SCD patients with permanent dentition; control₃, healthy children with deciduous or mixed dentition; and controlₚ, healthy children with permanent dentition.

**Periodontal evaluation**

All the examinations were performed at the Pediatric Hematology Clinic of Mersin University, Faculty of Medicine and Department of Pediatrics during morning hours after overnight fasting. All clinical periodontal and dental measurements were performed by the same experienced examiner (M.Ö.T.). Unstimulated saliva was collected for a period of 10 min, and its volume was recorded. Dental examinations were performed using the Williams periodontal probe (Henry Schein Inc., Melville, NY, USA) and a dental mirror in daylight and an additional portable light source (Keeler Instruments Inc. Windsor, UK). Clinical measurements of periodontal parameters, including plaque index (PI) (15), gingival index (GI) (16), bleeding on probing (BOP) (17), and probing pocket depth (PPD) were recorded. In addition, nasal or oral breathing status of the children was evaluated using a dental mirror.

Gingival enlargement was assessed using hyperplastic index (HI) (18). This index has been used frequently in recent studies (19,20). After examination, the periodontal health status of the subjects was evaluated using a biofilm-gingival interface (BGI) classification system (21).
Analysis of intra-examiner reproducibility
The intragroup reproducibility for all the data (PI, GI, PPD, HI) was determined by the examiner (MÖT) by assessing six patients. Each patient was evaluated twice during one visit over a 1-h interval, and all data measurements were repeated in these subjects in a blinded manner for the first assessment. The reproducibility was defined by a Kappa score. Kappa scores were calculated with non-weighted Kappa index with a 95% confidence interval (95% CI) and reported as 0.82, 0.83, 0.80, and 0.85 for PI, GI, PPD, and HI, respectively.

Histopathological evaluation
Since physical trauma, ache, and stress may induce painful vaso-oclusive SCD crisis, histopathological evaluation of the enlarged gingiva was performed for only one child (a 9 year-old girl) who needed extraction of the left maxillary first molar because of gingival enlargement and deep caries and volunteered for gingival biopsy (Fig. 1a). The gingival tissue biopsy (sized 0.4 x 0.3 x 0.1 mm) was obtained during the tooth extraction. Additionally, as control, a healthy gingival sample (sized 0.2 x 0.3 x 0.1 mm) was obtained from a systemically healthy 9 year-old child during maxillary first deciduous molar tooth extraction because of deep occlusal caries.

The gingival tissue samples were embedded in paraffin and stained with hematoxylin-eosin (HE) and evaluated microscopically for inflammatory and fibrous changes in gingival tissue.

Sample size calculation
According to the 2013 data of the Turkish Statistical Institute, 430,836 children aged 5-18 years are present in the Mersin province (www.tuik.gov.tr/ilGostergeleri/iller/MERSIN.pdf). Of these, 60 children were diagnosed and registered with SCD by the hospitals present in Mersin. Therefore, the proportion of children having SCD was calculated as 0.014%; 49 of these 60 children with SCD were included in the present study.

The relevance of the study was calculated using a package program (NCSS/PASS 2011, Dawson Edition; NCSS, Kaysville, UT, USA) for α = 0.01; β/α ratio = 1 using the means and standard deviations (SD) of all the periodontal parameters. Post hoc power of the present study for HI was determined to be 0.97 (effect size = 0.78, α = 0.05) in SCD and control groups. The post hoc power of the permanent and deciduous/mixed dentition subgroups for HI was 0.88 (effect size = 0.83, α = 0.05), and 0.78 (effect size = 0.78, α = 0.05), respectively.

Statistical analyses
All comparisons were made between the SCD and control groups, the matched subgroups SCD<sub>d</sub> and control<sub>d</sub>, SCD<sub>p</sub> and control<sub>p</sub>, were compared statistically for study parameters as well. The sex differences between the groups were determined by using the Chi-square test. Normality of the data was determined by the Shapiro-Wilk’s test. The normally distributed data were age, PI, GI, and BOP. These normally distributed data were expressed as mean ± SD and group comparisons were made using indepen-
Results

Clinical findings

Demographic and oral health related parameters of the study groups are shown in Table 1. There were no statistically significant differences between the SCD and control groups and between the matched subgroups with regard to age, gender, number of teeth, periodontal health status, and unstimulated salivary volume ($P > 0.05$). There were no subjects with periodontitis in the study groups. The majority of the study participants had gingivitis.

In the SCD group, 28 patients (57.1%) had the SS genotype, and 21 patients (42.9%) had the SB genotype. The frequencies of the evaluated medical parameters of the SCD groups are given in Table 2. Statistically significant difference between the SCD$_d$ and SCD$_p$ was noted with regard to the history of acute chest syndrome ($P < 0.05$). There were no statistically significant differences between the study groups and between the subgroups with regard to all of the periodontal parameters except HI and gingival enlargement frequency (GE) ($P < 0.05$). GE frequency and the mean ± SD values of the PI, GI and BOP, HI, and PPD in the study groups are shown in Table 2.

Table 1 Demographic and dental characteristics of the study groups

<table>
<thead>
<tr>
<th>Demographic and dental characteristics</th>
<th>SCD $n = 49$</th>
<th>Control $n = 39$</th>
<th>SCD$_d$ $n = 17$</th>
<th>Control$_d$ $n = 20$</th>
<th>SCD$_p$ $n = 32$</th>
<th>Control$_p$ $n = 19$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>12.6 ± 3.34</td>
<td>11.3 ± 3.44</td>
<td>9 ± 2.23</td>
<td>8.55 ± 2.04</td>
<td>14.5 ± 1.96</td>
<td>14.26 ± 1.76</td>
</tr>
<tr>
<td>Sex (F/M) ($n$)</td>
<td>18/31</td>
<td>19/20</td>
<td>6/11</td>
<td>8/12</td>
<td>12/20</td>
<td>11/8</td>
</tr>
<tr>
<td>Number of teeth</td>
<td>Median (min-max)</td>
<td>26 (15-28)</td>
<td>24 (20-28)</td>
<td>24 (15-28)</td>
<td>23 (20-25)</td>
<td>27 (22-28)</td>
</tr>
<tr>
<td>BGI ($n$) (BGI-H/BGI-G)</td>
<td>6/43</td>
<td>5/34</td>
<td>2/15</td>
<td>4/16</td>
<td>4/28</td>
<td>1/18</td>
</tr>
</tbody>
</table>


Table 2 The clinical characteristics of the sickle cell disease (SCD) groups

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>SCD $n = 49$</th>
<th>SCD$_d$ $n = 17$</th>
<th>SCD$_p$ $n = 32$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of SCD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbSS</td>
<td>28 (57.1)</td>
<td>11 (64.7)</td>
<td>17 (53.1)</td>
</tr>
<tr>
<td>HbSB</td>
<td>21 (42.9)</td>
<td>6 (35.3)</td>
<td>15 (46.9)</td>
</tr>
<tr>
<td>Frequency of vaso-occlusive crisis in the last year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32 (65.3)</td>
<td>10 (58.8)</td>
<td>22 (68.8)</td>
</tr>
<tr>
<td>1-5</td>
<td>14 (28.6)</td>
<td>6 (35.3)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>3 (6.1)</td>
<td>1 (5.9)</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td>Frequency of blood transfusion in the last year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25 (51.1)</td>
<td>5 (29.4)</td>
<td>20 (62.5)</td>
</tr>
<tr>
<td>1-5</td>
<td>18 (36.7)</td>
<td>10 (58.8)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>6 (12.2)</td>
<td>2 (11.8)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>History of acute chest syndrome</td>
<td>15 (30.6)</td>
<td>2 (11.8)</td>
<td>13 (40.6)*</td>
</tr>
<tr>
<td>History of splenic sequestration</td>
<td>13 (26.5)</td>
<td>5 (29.4)</td>
<td>8 (25)</td>
</tr>
<tr>
<td>History of splenectomy</td>
<td>11 (22.4)</td>
<td>2 (11.8)</td>
<td>9 (28.1)</td>
</tr>
<tr>
<td>History of stroke</td>
<td>5 (10.2)</td>
<td>1 (5.9)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Usage of hydroxyurea</td>
<td>40 (81.6)</td>
<td>14 (82.4)</td>
<td>26 (81.3)</td>
</tr>
<tr>
<td>Usage of chelating agent</td>
<td>9 (18.4)</td>
<td>5 (29.4)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>Usage of penicillin</td>
<td>45 (91.8)</td>
<td>16 (94.1)</td>
<td>29 (90.6)</td>
</tr>
<tr>
<td>Usage of folic acid</td>
<td>49 (100)</td>
<td>17 (100)</td>
<td>32 (100)</td>
</tr>
</tbody>
</table>

SCD$_d$: SCD patients with deciduous or mixed dentition, SCD$_p$: SCD patients with permanent dentition. *: statistically significant differences between the SCD$_d$ and the SCD$_p$ subgroups ($P < 0.05$, Chi-square test)

dent sample t-test. The other non-normally distributed data were presented as median (min-max) values. The Mann-Whitney U test was used for group comparisons for the non-normally distributed data. Because the data regarding medical parameters in SCD group were not distributed normally, Spearman correlation coefficient (rho) was used to analyze the relationships between periodontal and medical parameters in the whole SCD group. Multivariate analysis of variance (MANOVA) was used to investigate of the interaction of the independent variables; $P < 0.001$ and $P < 0.05$ were considered to be significant. All statistical calculations were performed using a software package program (SPSS 10, SPSS Inc., Chicago, IL, USA).
Table 3 Intergroup comparisons of the clinical parameters; plaque index (PI), gingival index (GI), probing pocket depth (PPD), bleeding on probing (BOP), gingival enlargement (GE) frequency, and hyperplastic index (HI)

<table>
<thead>
<tr>
<th>Clinical Parameters</th>
<th>SCD</th>
<th>Control</th>
<th>SCDd</th>
<th>Controld</th>
<th>SCDp</th>
<th>Controlp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 49</td>
<td>n = 39</td>
<td>n = 17</td>
<td>n = 20</td>
<td>n = 32</td>
<td>n = 19</td>
</tr>
<tr>
<td>GE frequency (n %)</td>
<td>27 (55.1)</td>
<td>6 (15.3)**a</td>
<td>10 (58.8)</td>
<td>4 (20) a</td>
<td>17 (53.1)</td>
<td>2 (10.5)‡a</td>
</tr>
<tr>
<td>HI (mean ± SD)</td>
<td>0.29 ± 0.38</td>
<td>0.07 ± 0.19**a</td>
<td>0.37 ± 0.46</td>
<td>0.09 ± 0.24b</td>
<td>0.24 ± 0.34</td>
<td>0.03 ± 0.11b</td>
</tr>
<tr>
<td>PI (mean ± SD)</td>
<td>1.14 ± 1.04</td>
<td>1.10 ± 0.54</td>
<td>1.18 ± 0.38</td>
<td>1.21 ± 0.63</td>
<td>1.11 ± 0.59</td>
<td>0.95 ± 0.37</td>
</tr>
<tr>
<td>GI (mean ± SD)</td>
<td>0.96 ± 0.54</td>
<td>0.87 ± 0.47</td>
<td>0.98 ± 0.52</td>
<td>0.98 ± 0.50</td>
<td>0.95 ± 0.57</td>
<td>0.72 ± 0.39</td>
</tr>
<tr>
<td>PPD Median (min-max)</td>
<td>1.19 (1-1.88)</td>
<td>1.17 (1-2.15)</td>
<td>1.14 (1-1.79)</td>
<td>1.16 (1-2.15)</td>
<td>1.23 (1-1.88)</td>
<td>1.18 (1-2.02)</td>
</tr>
<tr>
<td>BOP % (mean ± SD)</td>
<td>40 ± 24</td>
<td>35 ± 22</td>
<td>40 ± 26</td>
<td>38 ± 24</td>
<td>41 ± 23</td>
<td>31 ± 19</td>
</tr>
<tr>
<td>Unstimulated Salivary volume (mean ± SD)</td>
<td>2.95 ± 2.08</td>
<td>3.12 ± 3.06</td>
<td>2.86 ± 2.43</td>
<td>3.02 ± 1.82</td>
<td>3.60 ± 3.91</td>
<td>2.47 ± 1.03</td>
</tr>
</tbody>
</table>

SCD: Sickle cell disease, SCDd: SCD patients with deciduous or mixed dentition, SCDp: SCD patients with permanent dentition, Controld: healthy children with deciduous or mixed dentition, Controlp: healthy children with permanent dentition. *: significant differences between the SCD and the Control groups (P < 0.05), **: significant differences between the SCD and the Control groups (P < 0.001), §: significant differences between the SCDd and the Controld groups (P < 0.05), ‡: significant differences between the SCDp and the Controlp groups (P < 0.05), a: Chi-Square test, b: Mann-Whitney U test

Table 4 The gender distribution, location of GE and mean ± SD values of plaque index, gingival index and bleeding of probing in enlargement region (PI-E, GI-E, BOP-E) of the subjects having GE in the groups and subgroups

<table>
<thead>
<tr>
<th>Characteristics of GE</th>
<th>SCD</th>
<th>Control</th>
<th>SCDd</th>
<th>Controld</th>
<th>SCDp</th>
<th>Controlp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 27</td>
<td>n = 6</td>
<td>n = 10</td>
<td>n = 4</td>
<td>n = 17</td>
<td>n = 2</td>
</tr>
<tr>
<td>Gender distribution n (F/M)</td>
<td>27 (8/19)</td>
<td>6 (1/5)</td>
<td>10 (2/8)</td>
<td>0/4</td>
<td>17 (6/11)</td>
<td>1/1</td>
</tr>
<tr>
<td>Location of GE (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>16</td>
<td>*</td>
<td>10</td>
<td>0/4</td>
<td>17</td>
<td>1/1</td>
</tr>
<tr>
<td>Posterior</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Whole mouth</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>PI-E (mean ± SD)</td>
<td>1.11 ± 1.03</td>
<td>1.09 ± 0.69</td>
<td>1.20 ± 0.26</td>
<td>1.24 ± 0.59</td>
<td>1.13 ± 0.52</td>
<td>1.16 ± 0.55</td>
</tr>
<tr>
<td>GI-E (mean ± SD)</td>
<td>1.07 ± 0.45</td>
<td>1.52 ± 0.35</td>
<td>1.02 ± 0.32</td>
<td>1.47 ± 0.48</td>
<td>1.10 ± 0.51</td>
<td>1.61 ± 0.39</td>
</tr>
<tr>
<td>BOP-E (%) (mean ± SD)</td>
<td>47.8 ± 21.8</td>
<td>49.3 ± 19.5</td>
<td>41.6 ± 25.2</td>
<td>51.5 ± 14.1</td>
<td>51.4 ± 19.5</td>
<td>47.0 ± 22.6</td>
</tr>
<tr>
<td>PPD-E Median (min-max)</td>
<td>1.30 (1-2.57)</td>
<td>1 (1-1.70)</td>
<td>1.40 (1-2.57)</td>
<td>1 (1-1.30)</td>
<td>1.25 (1-2.50)</td>
<td>1.35 (1-1.70)</td>
</tr>
</tbody>
</table>

SCD: Sickle cell disease, SCDd: SCD patients with deciduous or mixed dentition, SCDp: SCD patients with permanent dentition. Controld: healthy children with deciduous or mixed dentition, Controlp: healthy children with permanent dentition, *: statistically significant differences between the SCD and the Control groups (P < 0.001, Chi-Square test), §: statistically significant differences between the SCDd and the Controld groups (P < 0.05, Chi-Square test), ‡: statistically significant differences between the SCDp and the Controlp groups (P < 0.05, Chi-Square test)

Table 3. GE was detected in 27 children (55.1%) in the SCD group and 6 children (15.4%) in the control group (P < 0.001). GE was observed more frequently in boys than in girls in both study groups and the dentition subgroups (P < 0.05) (Table 4). The gender distribution, location of GE and mean ± SD values of PI, GI, PPD, and BOP in enlargement region (PI-E, GI-E, PPD-E, BOP-E) of the subjects having GE in the groups and subgroups are shown in Table 4. However, there were no statistically significant differences between the groups and subgroups regarding PI, GI, PPD, and the BOP values of the enlargement regions. The PPD-E values were higher in the SCD group and subgroups except the SCDp group (Table 4). The location of the tooth with GE in the SCD group was significantly different from that in the control group (P < 0.001); moreover, this significance was valid for the subgroups as well (P < 0.05).

The GE observed in SCD group reached from gingival margin to the muco-gingival junction. The thickened and shelf-like contour of the marginal gingiva in this region was remarkable in several SCD patients. The clinical appearances of the three SCD patients (9-11 years old) are presented in Fig. 1a-c. The enlargements were especially in the horizontal direction and had most a more pronounced fibrotic appearance in children with SCD.
Figure 1d represents the knife-edge contour of healthy marginal gingiva in a systemically healthy 9 year-old child from the control group (Fig. 1a-c).

When the correlations between the medical and the dental/periodontal parameters were assessed in the whole SCD group, there were significant positive correlations between the history of splenic sequestration and HI ($\rho = 0.368$, $P < 0.01$), between the history of acute chest syndrome and the number of teeth ($\rho = 0.363$, $P < 0.01$), and between the presence of GE and some periodontal parameters like BGI ($\rho = 0.289$, $P < 0.01$) and BOP ($\rho = 0.341$, $P < 0.05$). In addition, HI values were positively associated with GI ($\rho = 0.295$, $P < 0.05$) and BOP ($\rho = 0.315$, $P < 0.05$).

When the effects of all parameters were evaluated together, the interaction between groups and age groups was not statistically significant ($P > 0.05$), the differences between the groups were also not significant ($P > 0.05$). The number of teeth was the only significantly different variable between the age groups in MANOVA analysis ($P < 0.05$).

**Histopathological findings**

Paraffin-embedded samples stained with HE from the GE region showed hyperplastic epithelium and slight lymphocyte and plasma cell infiltration in a fibrotic connective tissue stroma of the gingival mucosa (Fig. 2a).

![Histological findings of gingival tissue samples harvested from gingival enlargement regions of a child with sickle cell disease.](image)

(a) Hyperplastic epithelium (white arrow) and lymphocyte and plasma cell infiltration (black arrow) in a fibrotic stroma (HE, 100×), (b) Russell bodies (arrow) are among the plasma cells and lymphocytes (HE, 400×), (c) Intraepithelial vessels filled with erythrocytes (arrow) and vessels entering the epithelium (HE, 100×), (d) Abnormally shaped erythrocytes are in some vessels (HE, 400×).

Figure 3 Hyalinized, fibrotic, dense scar-like stroma (arrow) of the gingival mucosa (HE, 100×).

Figure 4 Histopathological findings of gingival tissue samples of a systemically and periodontally healthy child.

Figure 5 Panoramic radiographs of patients with sickle cell disease who underwent gingival biopsy.

Focal dense scar-like fibrotic areas in connective tissue were noted. Some Russell bodies were also seen among the chronic mononuclear inflammatory cells (Fig. 2b). Some of the sections also suggested that blood vessels filled with erythrocytes entered the epithelium (Fig. 2c), especially in the basal layer. The shape of erythrocytes was abnormal and some of them were seen in blood vessels, while others were seen in the erythrocyte extravasation areas (Fig. 2d). Hyalinization and fibrosis (arrow) were detected in the connective tissue of the gingival mucosa (Fig. 3). Figure 4 shows the histopathologic appearance...
of a healthy gingiva from a systemically healthy child. A small number of leukocytes, normally structured vessels and connective tissue were observed in healthy gingival tissue. There were no radiographic abnormalities except for deep caries in the left maxillary first molar (Fig. 5).

**Discussion**

In the present study, we evaluated the periodontal health status in children with SCD and compared it to that of healthy children. To our knowledge, this is the first study evaluating the GE status in addition to periodontal health parameters in a group of children with SCD. Our findings revealed that there was a significant difference between children with SCD and healthy controls regarding the frequency and location of GE; however, there were no differences between the groups in terms of other periodontal parameters.

There are few studies in the literature associated with the periodontal health status of subjects with SCD (10-13,22-25). Previous studies focusing on the periodontal health status of patients with SCD are presented in Table 5. Two of these studies were conducted in children (10,25). The majority of these studies reported that there were no significant differences between SCD patients and healthy controls in terms of periodontal parameters (12,13,22-25). In agreement with those results, the oral health status of children with SCD was similar to those of their healthy counterparts in our study. The majority of individuals in both groups had gingivitis. Veiga et al. (25) reported that children with SCD had gingivitis with high levels of dental plaque and gingival bleeding similar to that observed in healthy children. In another study, higher GI values were observed in children with SCD compared to their healthy counterparts (23). Although the GI values of the SCD group were slightly higher than those of the control group, the difference was not significant in the present study.

GE was observed in a large number of patients with SCD (27 children, 55.1%), whereas it was noted in only 6 (15.4%) participants in the control group. Moreover, the whole-mouth GE was observed in 10 patients in the SCD group, whereas it was not observed in the control group.

There was only one case report in the literature regarding GE in SCD patients (7). The case report presented a 14 year-old Afro-Trinidadian boy with SCA having enlarged mandibular gingiva. It was reported that histopathologic assessment of the gingiva showed a hemorrhagic and fibrotic lamina propria with inflammatory cell infiltration.

The gingival samples harvested from the GE regions of a subject with SCD were evaluated histopathologically (Figs. 2, 3). Similar to the results of the case report
mentioned above (7), we observed focal dense scar-like areas and fibrosis in the connective tissue stroma (Fig. 3). Fibrosis was characterized as an increased accumulation of fibroblasts, collagen, and other extracellular matrix components (26). The classical features of GE include greatly elongated epithelial rete pegs extending deep into the connective tissue as well as gingival lesions containing fibrotic or expanded connective tissue with characteristic levels of inflammation (27). The histopathological observations in the present study conform to the findings associated with gingival overgrowth.

In addition, it was observed that blood vessels filled with abnormally shaped erythrocytes in close proximity to the epithelium were present. Russell bodies were observed among the chronic mononuclear inflammatory cells (Fig. 2b). Abnormal plasma cells called Mott cells, containing immunoglobulin aggregates termed Russell bodies, have been found in non-secretory myeloma, inflammatory diseases, and autoimmune disorders (28). Furthermore, histopathologic signs of chronic inflammation include elongated rete pegs, fibrosis, and abnormal plasma cells, Russell bodies or abnormally shaped erythrocytes resulting from sickling and vaso-occlusion were not observed in histopathologic evaluation of the healthy gingival sample (Fig. 4). These findings give rise to the notion that the gingiva in patients with SCD was affected by chronic sickling and inflammation.

All patients with SCD in this study were administered 1 mg folic acid daily. This approach aims to keep a stable hemoglobin level. In addition, folate medication in SCD patients prevents hyperhomocysteinemia and thrombotic events leading to painful crises (29). The GE observed in the SCD group was mild. This may be as a result of daily folic acid intake by SCD patients. It was reported that systemic folic acid supplementation decreases phenytoin induced gingival overgrowth (30). Similarly, daily folic acid supplementation may be repressing the GE caused by chronic inflammation and sickling observed in SCD.

Patients with SCD are more susceptible to pulmonary infections and bacteremia. The etiologies of these infections involve functional defects in spleen, and tissue damage resulting from SCD. Therefore, antibiotics are essential to avoid infections in patients with SCD (31). Since periodontal diseases are chronic inflammatory and infectious diseases, the administration of systemic antibiotics reduces periodontal inflammation and improves clinical outcomes (32). Systemic amoxicillin and metronidazole combination is an effective adjunctive treatment along with mechanical periodontal therapy for periodontal diseases (32). Conversely, systemic penicillin regimen by itself cannot eliminate periodontal infection. In the present study, children with SCD who were administered penicillin prophylactically were included in the study 30 days after antibiotic usage in order to exclude the effects of penicillin on periodontal inflammation (14).

Puberty produces some changes in the physical appearance and behavior of adolescents. Sex steroid hormones have direct and indirect effects on proliferation, differentiation, and growth of keratinocytes and fibroblasts in the gingiva. During puberty, under the influence of sex hormones, periodontal tissues may mount an exaggerated response to local factors (33). Two cases were reported in the literature linking GE to puberty (34,35). In contrast, it was reported that hypogonadism, endocrine organ dysfunction, delayed growth, and pubertal development are frequently observed in children and adolescents with SCD (36). In addition, in another study conducted on the same population as this study, growth and pubertal development retardation were found to be more frequent in children with SCD compared to their healthy counterparts (37). Although the pubertal status of the participants was not assessed in this study, delayed sexual development has been recorded in their medical records for most of the SCD patients participating in this study.

Adenoid hypertrophy and tonsillitis are common in patients with SCD. It has been speculated that recurrent upper respiratory tract infections and chronic inflammation cause adenotonsillar hypertrophy. They are also associated with mouth breathing and hypoxia which trigger sickling, inflammation, and vaso-occlusive crises (38).

The nasal or oral respiratory status of the participants was evaluated using a dental mirror in this study. We did not observe mouth breathing in any subjects. In addition, the upper and lower lips of the subjects closed in the rest position. Since xerostomia and mouth breathing may increase dental plaque accumulation and induce gingival changes, the unstimulated salivary volume of the subjects was also evaluated. No differences were observed between the SCD and the control groups and subgroups regarding PI and salivary volume.

The present study has several limitations. The gingival tissue samples were not obtained from all the study participants. Histopathological evaluations of the gingiva were performed in only one subject from each group for ethical reasons. Furthermore, evaluating the effects of adenoid hypertrophy on GE was not possible owing to the lack of adenoidal examination in this study.

The results of the present study revealed that although periodontal health and oral hygiene status were similar to those of their healthy counterparts, marginal GE was observed more frequently in children with SCD. There-
fore, we conclude that SCD may predispose children to GE and that gingival tissues are affected by chronic inflammation and hemoglobin sickling that is also seen in SCD. Furthermore, folic acid supplementation may be repress GE observed in children with SCD.

Acknowledgments

The authors are grateful to The Scientific and Technological Research Council of Turkey (TUBITAK) for supporting the study (project number 113S271).

Conflict of interest

The authors declare that they have no conflicts of interest related to this study.

References


