DOI: 10.1111/j.1365-263X.2007.00911.x

The changes in the T-lymphocyte subsets in a population of Turkish children with puberty gingivitis

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International Journal of Paediatric Dentistry 2009; 19: 206–212

Objective. The aim of the study was to investigate the number of CD4 and CD8 T lymphocytes, analyse subjects with gingivitis and those without, and determine the role of T lymphocytes in the pathobiology of puberty gingivitis.

Material and methods. Fifty individuals with and without puberty gingivitis were recruited for this study. The $CD4^+$ and $CD8^+$ T-lymphocyte counts were determined using flow cytometry on the biopsy samples, and the $CD4^+/CD8^+$ ratio was calculated. At the same time, periodontal index scores were recorded to assess the periodontal status. Acquired data were analysed statistically using a paired *t*-test to compare laboratory values obtained before and after the treatment in individuals with puberty gingivitis and disease-free individuals. In addition, Pearson's correlation analysis was performed to investigate the relation between laboratory values and clinical measurements.

Results. The $CD4^+/CD8$ ratio in gingival tissues obtained from test group was significantly higher ($P < 0.05$) than that found in the gingival tissue obtained from control group. We found that the $CD4^+$ and $CD8^+$ lymphocyte counts continued to increase significantly ($P < 0.001$) and the $CD4^+/CD8^+$ ratio continued to drop significantly ($P < 0.05$) after treatment in test group.

Conclusions. T lymphocytes could play a significant role in the pathobiology of puberty gingivitis

Introduction

Periodontal diseases are the most common diseases in the population. The classic periodontal diseases are gingivitis and periodontitis; there are, however, numerous and distinct types of both diseases. These types of diseases are distinguished by age of onset, clinical appearance, rate of disease progression, pathogenic microbial flora, and systemic influences[1].

Gingivitis may be divided into three progressive stages, progressing from normal gingiva to initial gingival lesion to early gingival lesion and finally to the established gingival lesion. Most gingivitis lesions are transient or persistent but not progressive[2].

Gingivitis may not be precursor of attachment loss and alveolar bone loss. Some gingivitis lesions do progress to periodontitis. Gingivitis in children is different from adult gingivitis by virtue of a different microflora and altered immunocytes. Clinical signs may be delayed in children because of a predominately T-lymphocyte response. Page[3], however, has emphasized that gingivitis should be classified as a disease.

It is well known that the virulence factors of microbial dental plaque are the main causes of gingivitis. In health, there is a balance, between these virulence factors and the host immune system. Yet, an imbalance in favour of the virulence factors may lead to the occurrence of the disease. Defending mechanism against infection may be local or systemic, specific or nonspecific, and humoral or cellular[4]. Pathology can be a consequence of the immune response in gingivitis[5]. Gingival diseases provide an important model for investigation of the pathological potential of human cellular infiltrates[6].

Although we have come across many studies dealing with diseases affecting oral cavity and T-lymphocyte levels while examining the related literature[7–10], no study has been encountered regarding the local immunoregulator cells in

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the patients with puberty gingivitis. The aim of this study was to investigate the number of CD4 and CD8 T lymphocytes, analyse subjects with gingivitis and those without, and determine the role of T lymphocytes in the pathobiology of puberty gingivitis.

Materials and methods

The present investigation was conducted using 50 volunteers (23 females and 27 males). According to study protocol, the subjects were divided equally into two groups (test and control). The volunteers were recruited from patients who had been referred to the Faculty of Dentistry at Atatürk University in Erzurum, Turkey, for periodontal and dental treatment. The volunteers were informed about the purpose and the methods of the study and all consented to participate.

The 50 volunteers were consecutively selected. The following inclusion criteria were applied. All the volunteers are in their puberty and had not received periodontal treatment or been treated with an antibiotic for any medical or dental reason for 3 months prior to their recruitment into the study. The volunteers had to have no history of systemic disease, such as diabetes, leukaemia, metabolic bone disease, or epilepsy. Volunteers with pubertal gingivitis should satisfy the following criteria: (i) have a minimum of 28 teeth; (ii) display clinical signs of gingivitis, namely gingival bleeding, changes in gingival colour, gingival contour, position, and surface texture in the gingivae; (iii) display attachment loss of less than 3 mm; (iv) have no pockets greater than 3 mm in depth; and (v) show no radiographic evidence of bone loss. For healthy volunteers, the inclusion criteria are (i) have a minimum of 28 teeth; (ii) have no clinical signs of gingivitis; (iii) display attachment loss of less than 3 mm; (iv) have no pockets greater than 3 mm in depth; and (v) show no radiographic evidence of bone loss.

In addition, occlusal adaptation conditions, such as open bite on anterior teeth, tongue thrust, lip biting, occupation-related peculiarities (holding nails or pins between the teeth, for example), and tipping of adjacent teeth into an extraction site resulting in occlusal imbalances, were considered.

Skeletal age and skeletal maturity stage were determined from hand–wrist radiographs using the method outlined in the atlas of Greulich and Pyle[11] and the Fishman's system[12]. Individuals who had not reached puberty or were at the end of their puberty were excluded from this study.

The clinical evaluation consisted of the plaque index (PI)[13], the gingival index (GI)[14], and probing pocket depths. The scores and measurements were made by the same periodontist in the Department of Periodontology, Atatürk University. All the clinical measurements in each volunteer were performed using William's periodontal probe (Hu-Friedy Manufacturing Inc., Chicago, IL, USA) on the gingival area adjacent to the teeth.

According to index scores, volunteers were divided into two groups: test group consisted of 25 volunteers with gingivitis, and control group consisted of 25 volunteers with healthy gingivae. The volunteers control group were recruited from patients who had been referred to our faculty for various dental reasons, such as orthodontic treatment. None of the control group volunteers had periodontal disease.

All volunteers had been instructed to apply fluoride toothpaste and to brush their teeth using the Bass methods for 3 min at least twice a day before biopsies were taken. Biopsy samples were taken just before initial periodontal treatment, and the biopsy samples were repeated in the third week following the treatment in test group. Biopsy samples (1.5–2 mm^2) were taken from the gingival pocket wall tissues at different affected sites of the same tooth. Biopsy samples were taken from sites of tooth extraction in patients who were undergoing treatment for other dental problems. No treatment was performed on the control group.

The numbers of $CD4^+$ and $CD8^+$ T lymphocytes in the biopsy samples were determined using flow cytometry. After collection, the biopsy samples were put into a phosphate buffer solution. Samples were then cut into very small pieces with a scalpel. The cut tissues were transformed into a suspension by using certain pore-size filters. A flow cytometer (Coulter EPICS®XL-MCL, Beckman Coulter Inc., Fullerton, CA, USA) was used for lymphocytes subsets analysis. The computer-assisted evaluation

was made with a commercially available software program (Coulter System II software version 2.0).

The lymphocytes were gated using volume and site scatter, and the specific fluorescence was quantified with a four-quadrant setting of a two-colour fluorescence dot blot. The absolute number of T lymphocytes was counted, and the relative proportion of each T-lymphocyte subpopulation was calculated.

Statistical analysis

The data were analysed statistically by paired *t*-test to compare the laboratory values obtained before and after the treatment in individuals with puberty gingivitis (test group) and individuals without (control group), and Pearson's correlation analysis to determine the relationship between the laboratory values and clinical measurements, using SPSS for Windows, Release 9.0.0 (version 11.0).

Results

The present investigation was conducted using 50 volunteers divided equally into two groups (test and control). The ages ranged from 12 to 15 years with a mean age of 13.4 ± 1.02 years. The test group who had been diagnosed with puberty gingivitis was made up of 11 females and 14 males, whose ages varied from 13 to 16 years with a mean age of 13.68 ± 1.40 years, and the control group who did not have puberty gingivitis were composed of 12 females and 13 males. The ages ranged from 12 to 15 years with a mean age of 13.05 ± 1.51 years.

This study was conducted using 23 female and 27 male volunteers. The group diagnosed with puberty gingivitis was composed of 11 female volunteers, whose age ranged from 12 to 14 years (mean age 12.78 ± 1.30 years), and 14 male volunteers, whose age ranged from 13 to 16 years (mean age 13.48 ± 1.35 years). The group without puberty gingivitis consisted of 12 female volunteers, whose age varied from 12 to 14 years (mean age 13.03 ± 1.27 years), and 13 males, whose age varied from 12 to 16 years (mean age 13.83 ± 1.37 years).

The laboratory results and associated statistical comparisons according to sex are given in Tables 1 and 2. There were no significant differences between the $CD4^+$, $CD8^+$ lymphocyte mean values, and $CD4^+/CD8^+$ lymphocyte ratio according to sex in the both groups (Table 1). But there were significant difference between $CD4^+$, $CD8^+$ lymphocyte mean values, and $CD4^+/CD8^+$ lymphocyte ratio in the before- and after-treatment of females and males in the test group (Table 2) ($P < 0.001$).

Table 1. Statistical comparison of mean values of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ ratio for both groups according to sex.

		Test group				Control group			
Period	**Lymphocytes**	***n***	**Sex**	**Mean ± SD**	***P***	***n***	**Sex**	**Mean ± SD**	***P***
Before treatment	$CD4^+$	11	Female	23.92 ± 4.17		12	Female	25.11 ± 6.15	
					> 0.05				> 0.05
		14	Male	22.54 ± 4.13		13	Male	24.19 ± 5.95	
	$CD8^+$	11	Female	13.72 ± 4.12		12	Female	15.87 ± 4.08	
					> 0.05				> 0.05
		14	Male	12.69 ± 4.08		13	Male	14.89 ± 4.11	
	$CD4^+/CD8^+$	11	Female	1.89 ± 1.31		12	Female	1.57 ± 1.27	
					> 0.05				> 0.05
		14	Male	1.67 ± 1.28		13	Male	1.56 ± 1.32	
After treatment	$CD4^+$	11	Female	28.06 ± 7.09		12	Male	25.11 ± 6.14	
					> 0.05				> 0.05
		14	Male	26.43 ± 7.15		13	Male	24.18 ± 6.11	
	$CD8^+$	11	Female	18.23 ± 3.18		12	Female	15.69 ± 4.07	
					< 0.05				> 0.05
		14	Male	16.21 ± 5.26		13	Male	14.68 ± 4.09	
	$CD4^+/CD8$	11	Female	1.58 ± 0.25		12	Female	1.59 ± 1.28	
					< 0.05				> 0.05
		14	Male	1.51 ± 0.33		13	Male	1.58 ± 1.28	

SD, standard deviation.

Table 2. Statistical comparison of mean values of CD4⁺, CD8⁺, and CD4⁺/CD8⁺ ratio before treatment (BT) and after treatment (AT) phases.

			CD4⁺			CD8⁺			CD4⁺/CD8⁺		
	n	Sex	BT Mean ± SD	AT Mean ± SD	*P*	BT Mean ± SD	AT Mean ± SD	*P*	BT Mean ± SD	AT Mean ± SD	*P*
Test	11	Female	23.92 ± 4.17	28.06 ± 7.09	< 0.001	13.72 ± 4.12	18.23 ± 3.18	< 0.001	1.89 ± 1.31	1.58 ± 0.25	< 0.001
Control	12	Female	25.11 ± 6.15	25.11 ± 6.14	> 0.05	15.87 ± 4.08	15.69 ± 4.07	> 0.05	1.57 ± 1.27	1.59 ± 1.28	> 0.05
Test	14	Male	22.54 ± 4.13	26.43 ± 7.15	< 0.001	12.69 ± 4.08	16.21 ± 5.26	< 0.001	1.67 ± 1.28	1.51 ± 0.33	< 0.01
Control	13	Male	24.19 ± 5.95	24.18 ± 6.11	> 0.05	14.89 ± 4.11	14.68 ± 4.09	> 0.05	1.56 ± 1.32	1.58 ± 1.28	> 0.05

SD, standard deviation.

Table 3. Statistical comparison of PI, GI, and PD value for the two groups.

			Gingival index		Plaque index		Probing depth (mm)	
Treatment phases	Groups	*n*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*
Before treatment	Test	25	2.16 ± 0.58		2.09 ± 0.50		1.68 ± 0.16	
				< 0.001		< 0.001		< 0.001
	Control	25	0.48 ± 0.60		0.68 ± 0.39		1.36 ± 0.12	
After treatment	Test	25	0.52 ± 0.33		0.46 ± 0.35		1.35 ± 0.12	
				> 0.05		> 0.05		> 0.05
	Control	25	0.46 ± 0.36		0.29 ± 0.29		1.31 ± 0.14	

GI, gingival index: PI, plaque index; PD, probing depth; SD, standard deviation.

Table 4. Statistical comparison of periodontal findings of test group in the before treatment and after treatment phases.

		Gingival index		Plaque index		Probing depth (mm)	
Treatment phases	*n*	Mean ± SD	*P*	Mean ± SD	*P*	Mean ± SD	*P*
Before treatment	25	2.16 ± 0.58		2.09 ± 0.50		1.68 ± 0.16	
			< 0.001		< 0.001		< 0.001
After treatment	25	0.52 ± 0.33		0.46 ± 0.35		1.35 ± 0.12	

The clinical periodontal findings of test group individuals before and after treatment and control group individuals are summarized in Tables 3 and 4. The mean before-treatment PI score of test group was significantly higher than that of control group ($P < 0.001$). A similar evaluation was made for GI and probing depth (PD) score. The before-treatment scores for GI and PD of test group individuals were significantly higher than those of control group individuals (Table 3). The PI, GI, and PD scores of test group individuals decreased noticeably following treatment ($P < 0.001$) (Table 4). Although the before-treatment scores for PI, GI, and PD of test group individuals were higher than those of control group individuals, this difference was not statistically significant ($P > 0.05$) (Table 3).

The mean relative number of $CD4^+$ and $CD8^+$ T-lymphocytes and $CD4^+/CD8^+$ ratios in gingival tissue obtained before and after treatment from test group individuals and control group individuals is given in Tables 5 and 6. The mean relative number of $CD4^+$ and $CD8^+$ T lymphocytes for test group at the beginning of the study was lower than that determined in control group individuals (Table 5). The before-treatment $CD4^+/CD8^+$ ratio in the test group individuals was significantly higher than that in the control group individuals ($P < 0.05$) (Table 5). After treatment, the mean relative number of $CD4^+$ and $CD8^+$ lymphocytes of test group individuals increased significantly ($P < 0.001$) (Table 5). This increase was associated with a significant ($P < 0.05$) fall in the $CD4^+/CD8^+$ ratio. The mean after-treatment relative

Table 5. Comparison of mean values of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio before treatment and after treatment phase.

			$CD4^+$		$CD8^+$		$CD4^+/CD8^+$	
Treatment phases	**Groups**	***n***	**Mean ± SD**	***P***	**Mean ± SD**	***P***	**Mean ± SD**	***P***
Before treatment	Test	25	23.56 ± 4.83		13.84 ± 1.63		1.76 ± 0.29	
				> 0.05		> 0.05		> 0.05
	Control	25	24.16 ± 4.89		15.86 ± 2.87		1.55 ± 2.81	
After treatment	Test	25	27.53 ± 4.25		17.69 ± 2.41		1.56 ± 0.31	
				> 0.05		> 0.05		> 0.05
	Control	25	24.16 ± 4.85		15.86 ± 2.73		1.54 ± 1.65	

SD, standard deviation.

Table 6. Comparison of mean values of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio for the test group before treatment and after treatment phase.

			$CD4^+$		$CD8^+$		$CD4^+/CD8^+$	
Treatment phases	**Group**	***n***	**Mean ± SD**	***P***	**Mean ± SD**	***P***	**Mean ± SD**	***P***
Before treatment	Test	25	23.56 ± 4.83		13.84 ± 1.63		1.76 ± 0.29	
				< 0.001		< 0.001		< 0.05
After treatment	Test	25	27.53 ± 4.25		17.69 ± 2.41		1.56 ± 0.31	

SD, standard deviation.

number of $CD4^+$ and $CD8^+$ T lymphocytes and the $CD4^+/CD8^+$ ratio in gingival tissues obtained from test group individuals was statistically significant (Table 6). There were, however, no statistical differences between the after-treatments $CD4^+/CD8^+$ ratios that were calculated for test group individuals and those calculated for control group individuals ($P > 0.05$) (Table 5).

Discussion

The change in T-lymphocyte subsets after applying periodontal treatment (initial periodontal treatment) in patients with puberty gingivitis was evaluated using laboratory and clinical findings.

Gingivitis starts in early childhood but its degree of severity increases with age[15]. The distribution of the ages where one observes a maximum prevalence of gingivitis corresponds to period of age where puberty occurs. The correlation between the degree of gingival inflammation and those indices that denote pubertal maturation suggests that a hormonal influence, concomitant with puberty, influences the gingival inflammatory process[16,17].

In this study, we planned to use the local $CD4^+/CD8^+$ ratio to identify which immunoregulatory mechanisms were operative in puberty gingivitis. To this end, we took biopsy samples from volunteers who did not have and who had symptoms of puberty gingivitis.

The $CD4^+$ and $CD8^+$ lymphocytes, which are among the effective cells of cellular immunity, have a central role in immunoregulation, because they can lead not only to cellular immune response but also to humoral immune response in nonspecific inflammatory reactions[18,19]. Although only the $CD4^+$ population was originally considered to subdivide into Th1 or Th2, subtypes of $CD8^+$ effector cells, called T1 and T2 or Tc1 and Tc2, have also been recognized[18–20]. Subtypes of $CD4^+$ and $CD8^+$ T cells exist in diseased periodontal tissues[6,7,18–22]. The ratio of $CD4^+$ cells to $CD8^+$ cells (CD4/CD8) is considered to be an important indicator for immune system functions[21,22]. A number of studies have compared the distribution of $CD4^+$ and $CD8^+$ T lymphocytes in periodontal tissue obtained from individuals suffering from rapidly progressing periodontitis and healthy volunteers. They reported that the $CD8^+$ lymphocyte count was lower in individuals with periodontal diseases than that in healthy individuals[23–25]. Furthermore, they reported that the $CD4^+$ lymphocyte count in these two

groups of individuals was not significantly different[24]. In this study, we found that both $CD4^+$ and $CD8^+$ lymphocyte counts in the volunteers with puberty gingivitis were lower than those in the healthy group.

Contradictory results have been obtained in studies on the character of the $CD4^+/CD8^+$ ratio in individuals with periodontal diseases[26–32]. Many investigators have reported that the $CD4^+/CD8^+$ ratio in diseased gingival tissue was lower than that found in healthy tissue[26,28–30], whereas others reported that this ratio increased[24,30,31]. On the other hand, some studies show that this ratio is the same in individuals with periodontal disease and healthy people[32]. In this study, we found that the $CD4^+/CD8^+$ ratio in gingival tissue obtained from volunteers with puberty gingivitis was greater than that calculated in healthy volunteers.

Okada *et al.*[33] found that T-lymphocyte subsets that were lower before treatment returned to normal after a well-applied curettage. It has also been reported that the $CD4^+$ T-lymphocyte count and the $CD4^+/CD8^+$ ratio are low and that the $CD8^+$ lymphocyte count is altered in periodontal lesions. Following treatment, the $CD4^+/CD8^+$ ratio and the $CD4^+$ T-lymphocyte count increase, whereas the $CD8^+$ T-lymphocyte count drops[34]. Similar to the results by Erciyas *et al.*[35], we found an increased number of $CD4^+$and $CD8^+$ T lymphocytes, and that their numbers continued to rise significantly after treatment. At the same time, the $CD4^+/CD8^+$ ratio continued to decrease after treatment. These observations imply that advanced cases of periodontal disease, in which a decreased CD4/CD8 ratio was seen, are in the process of healing[29]. There were no differences between $CD4^+$ and $CD8^+$ lymphocyte count and the $CD4^+/CD8^+$ ratio after treatment in the individuals with periodontal disease and the healthy volunteers. As a result, we found a negative correlation between clinical parameters and T-lymphocyte subsets. This finding confirms previously published data[35–37]. We found a statistical significant decrease in the severity scores of periodontal disease after treatment, and this result confirms the findings reported by others[35].

In some studies, a correlation between plaque index and T-lymphocyte subsets was not found[24,25,37,38], whereas in other studies, a negative correlation was reported between plaque index and the two $CD4^+$ and $CD8^+$ lymphocyte counts[35,38]. In this study, we found a negative relationship between $CD4^+$and $CD8^+$ lymphocyte counts and plaque index after treatment.

Other investigations have determined the correlation between gingival index and T-lymphocyte subsets[24,25,37]. Although Stashenko *et al.*[37] and Erciyas *et al.*[35] found a negative correlation between $CD4^+$ T-lymphocytes and gingival index, other researchers found no correlation between these two variables[24,25]. In this study, we found negative correlations between after-treatment GI and PD scores and the $CD4^+$ and $CD8^+$ lymphocyte count.

Conclusion

A peak in the severity of gingivitis at puberty occurs in individuals with inadequate oral hygiene. T lymphocytes appear to play a significant role in the pathobiology of puberty gingivitis.

What this paper adds

- The $CD4^+$ and $CD8^+$ T-lymphocyte counts in the volunteers with puberty gingivitis were lower than those in the healthy group.
- The $CD4^+/CD8^+$ ratio in the volunteers with puberty gingivitis was higher than that in the healthy group.
- There were increased number of $CD4^+$and $CD8^+$ T lymphocytes and decreased $CD4^+/CD8^+$ ratio after treatment.

Why this paper is important to paediatric dentists

- This paper demonstrates that T lymphocytes appear to play a significant role in the pathobiology of puberty gingivitis.

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