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The changes in T lymphocyte subsets following periodontal treatment in patients with chronic periodontitis

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Objective: The aim of this study was to determine whether there was any change in T-lymphocyte subsets in patients with chronic periodontitis after applying different periodontal treatment methods.

Patients and methods: Twenty-four patients with chronic periodontitis were included in the study. In every phase of the treatment (pretreatment, initial treatment, curettage and flap operations) the biopsy samples were taken from the gingival tissues at sites of chronic periodontitis. Then $CD4^+$ and $CD8^+$ lymphocyte and $CD4^+/CD8^+$ ratio values were determined using flow cytometry in the biopsy samples. At the same time, gingival pocket depth, Löe–Silness gingival index, and Silness–Löe plaque index scores were recorded to assess the periodontal status in patients. To determine the correlation between the clinical measurements and the laboratory results obtained before the treatment, after initial treatment, after curettage and after flap operations, we conducted an analysis using a paired *t*-test.

Results: Flow cytometry findings in the patients with chronic periodontitis showed that $CD4^+$ and $CD8^+$ lymphocyte values before treatment were under the normal value and the $CD4^+/CD8^+$ ratio was within the normal distribution interval. The $CD4^+/CD8^+$ ratio decreased postcurettage and postflap operation. This decrease was statistically significant ($p < 0.001$). The $CD4^+$ and $CD8^+$ lymphocyte values were increased postcurettage and postflap operation. This increase was also statistically significant ($p < 0.001$).

Conclusions: These findings suggest that local immune response was poor in the patients with chronic periodontitis. $CD4^+$ and $CD8^+$ T-lymphocytes could play a significant role in chronic periodontitis pathobiology.

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The term periodontitis is used to describe a group of multifactorial diseases that result in the progressive destruction of the structures that support the teeth within the jaws, the so-called attachment apparatus, which includes the periodontal ligament, cementum and alveolar bone. An inflammatory disease of the periodontal tissues initiated by microbial dental plaque can spread into periodontal ligament and alveolar bone by causing the destruction of the collagen fibers. If periodontitis is untreated, teeth can become loose and ultimately lost (1).

It is well known that the virulence factors of microbial dental plaque are the main causes of periodontal diseases. In health, there is a balance between these virulence factors and the host immune system, yet an imbalance in favor of the virulence factors may lead to the occurrence of the disease. Defence mechanisms against infection may be local or systemic, specific or non-specific, and humoral or cellular (2). Pathology can be a consequence of the immune response in periodontal diseases (3–10), and the diseases provide an important model for investigation of the pathologic potential of human cellular infiltrates (11).

$CD4^+$ and $CD8^+$ lymphocytes, which are some of the effector 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 non-specific inflammatory reactions (12, 13). 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 (5). Subtypes of $CD4^+$ and $CD8^+$ T cells exist in diseased periodontal tissues (6, 9–11, 14, 15) The ratio of $CD4^+$ cells to $CD8^+$ cells ($CD4^+/CD8^+$) is considered to be an important indicator for immune system functions (16).

Although there are many studies dealing with diseases affecting the oral cavity and T-lymphocyte levels (12, 13, 16–19), there are few studies regarding the local immunoregulator cells in patients with chronic periodontitis (20–22).

The aim of this study was to determine whether there was any change in T-lymphocyte subsets in patients with chronic periodontitis after applying different treatment methods.

Material and methods

Specimens of diseased gingival were obtained from patients between the ages of 35 and 50 years who were suffering from chronic periodontitis. They were treated with scaling and root planing before curettage and periodontal surgery. All of the subjects were chosen from those patients without a systemic disease or an oral disease except chronic periodontitis.

The exclusion criteria were the use of a drug that affects the oral flora (e.g. povidone-iodine, chlorhexidine, Listerine) or an immune system or inflammatory response in the 6 months before the assessment.

The patients were informed about the purpose and the method of the study, and they all agreed to participate.

Sample collection

Specimens were taken just before periodontal treatments (scaling and root planing, curettage, and periodontal surgery) from sites with advanced bone destruction and a probing depth of 5 mm or greater. The biopsy samples were repeated in the third week following the initial treatment, in the seventh week following the curettage, and in the eleventh week following the flap operation. In every phase of the treatment the biopsy samples (1.5–2 mm) were taken from the gingival pocket wall tissues at different diseased sites of the same tooth.

Adult subjects with chronic periodontitis were evaluated in the following four phases: before treatment, after initial treatment, after curettage and after flap surgery. In every phase of the treatment the $CD4^+$ lymphocyte values (normal range in peripheral blood 25–29%) (20), $CD8^+$ lymphocyte values (normal range in peripheral blood 19–48%) (23) and the $CD4^+/CD8^+$ ratio were determined using flow cytometry in the biopsy samples.

After clinical examination and measurements the patients were treated in accordance with different treatment procedures. Initial treatment (tooth surface cleaning, root surface smoothing), curettage and surgical operations were applied.

Flow cytometry evaluation

All biopsy samples taken were put into phosphate buffer solution. Then biopsy samples were cut into very small pieces with a scalpel. Broken tissues were transformed to suspension by filtering with certain sizes of filters. For lymphocyte subset analysis, a flow cytometer (FACS analyser, Becton & Dickinson, Mountain View, CA, USA) was used, and computer-assisted evaluation was made with a commercially available software program (Simultest Immune Monitoring Software/Becton & Dickinson, Mountain View, CA, USA).

With the use of volume and site scatter, lymphocytes were gated and specific fluorescence was quantified with a four-quadrant setting of a two-color fluorescence dot blot. The absolute as well as the relative (proportional) count of each lymphocyte subpopulation was calculated.

Clinical evaluation

Clinical evaluation consisted of the plaque index (24), gingival index (25), and probing pocket depths. The measurements were evaluated by the same periodontist carrying out the study in the Department of Periodontology, Atatürk University. All the clinical measurements were made using a manual periodontal probe (Williams' periodontal probe designed with 1, 2, 3, 5, 7, 9, and 10 mm calibrations) on the gingival area adjacent to the teeth in each patient.

The plaque index scores were recorded for the mesial, distal, buccal, and lingual surfaces for every tooth in each patient. The quantity of supragingival plaque was assessed at the cervical region of every tooth in each patient. Bleeding was recorded as positive if it occurred within 30 s after probing.

The numerical scores of the plaque index and gingival index were obtained according to the following formula: per person = sum of individual scores/number of teeth present for each person. Subsequently, a group score was calculated by adding together the individual scores and dividing the total into the number of patients included.

The mean of the plaque index, gingival index, and pocket depth were indicated as the mean of full mouth teeth from each patient for evaluating general oral hygiene and periodontal status.

Statistical analysis

Acquired data were evaluated statistically. The paired *t*-test was used to compare laboratory values obtained before the treatment, after initial treatment, after curettage, and after surgical operation. In addition, Pearson's correlation analysis was made to determine the relation between laboratory values and clinical measurements.

Results

The study was carried out on 24 patients (17 females and seven males) with chronic periodontitis. The age of the patients was from 35 to 50 and the mean age was 40.50 ± 4.72.

The laboratory results and associated statistical comparisons are given in Table 1. When evaluated statistically, a significant difference between $CD4^+$ lymphocyte mean values of pretreatment and post-treatment phases (initial treatment and curettage, and surgical operation) were observed ($p < 0.001$). A similar evaluation was also made for $CD8^+$ lymphocyte values and is given in Table 1. The difference between the $CD8^+$ lymphocyte value before treatment and the $CD8^+$ lymphocyte value post-treatment phases were statistically significant ($p < 0.001$), but the $CD8^+$ lymphocyte value was found to be lower than the normal value. Regarding the $CD4^+/CD8^+$ lymphocyte ratio, the difference between pretreatment and postcurettage was statistically significant ($p < 0.05$), and the difference between pretreatment and postsurgical values was found to be statistically significant ($p < 0.01$) (Table 1).

The clinical periodontal findings and statistical comparisons are given in Table 2. A decrease in plaque index values after initial treatment, after curettage, and after surgical operation by comparison to values before treatment was determined. This difference was statistically significant ($p < 0.001$). Similar findings were observed for gingival index scores and pocket depth.

A significant correlation was observed between gingival index value and $CD4^+$ ($p < 0.05$) and between plaque index and $CD8^+$ ($p < 0.05$) when the results of Pearson's correlation analysis and the correlation between T-lymphocyte subsets and clinical measurements were examined (Table 3).

Discussion

The change in T-lymphocyte subsets after applying different periodontal treatment methods in patients with chronic periodontitis was evaluated using laboratory and clinical findings.

Chronic periodontitis is the most common form of periodontitis and is slowly progressive and usually found in adults over the age of 35 years (26). Its progress varies from person to person and no systemic disorder is found in its etiology (27).

A lot of studies investigating the phenotypic features of cellular infiltrate in tissue with such variables as the type, activity, localization of periodontal disease and the treatment applied have shown that T-cells are dominant in the early period of disease, whereas B lymphocyte and plasma cells are dominant in its advanced period (28, 29).

There are some studies that show it is possible to examine $CD4^+$, $CD8^+$ and their subgroups using a variety of techniques (5, 7, 10). However, only flow cytometry was used in this study because this method is cost effective, objective, and faster, and it can easily isolate the lymphocytes from the tissues (23). In addition, the most important privilege of this method is its convertibility into a flow cytometry technique for quantitative analysis. Flow cytometry is the most commonly used and the most developed method of the two-color immunofluorescence techniques (30). Cells can be analyzed at the level of one cell through flow cytometry, and physical and biological features of the cells can be accurately and quantitatively measured (30). Because of all these qualities, we preferred the flow cytometry method in our study.

$CD4^+$ and $CD8^+$ lymphocytes, which are effective cells of cellular immunity, have the capability of cellular immune response and humoral immune response, as well as the ability to direct non-specific inflammatory reactions (4, 8). Therefore, they have a central role in immunoregulation. In our study, we planned to investigate the presence of the ratio of $CD4^+$ and $CD8^+$ lymphocytes at the local level with the intention of determining immunoregulation mechanisms that are effective in chronic periodontitis.

Table 1. Comparison of mean values of $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ ratio pretreatment and post-treatment phases (initial treatment and curettage, and surgical operation)

Treatment phase	$CD4^+$ lymphocyte mean ± SD	*p*	$CD8^+$ lymphocyte mean ± SD	*p*	$CD4^+/CD8^+$ ratio mean ± SD	*p*
Pretreatment	21.63 ± 8.17		8.58 ± 3.92		2.80 ± 1.26	
		> 0.05		< 0.01		> 0.05
Postinitial treatment	26.17 ± 8.33		11.75 ± 5.53		2.51 ± 1.03	
Pretreatment	21.63 ± 8.17		8.58 ± 3.92		2.80 ± 1.26	
		< 0.01		< 0.001		< 0.01
Postcurettage	30.00 ± 7.06		15.38 ± 4.39		2.00 ± 0.31	
Pretreatment	21.63 ± 8.17		8.58 ± 3.92		2.80 ± 1.26	
		< 0.001		< 0.001		< 0.01
Postflap operation	31.08 ± 5.25		17.00 ± 3.13		1.84 ± 0.16	
Postinitial treatment	26.17 ± 8.33		11.75 ± 5.53		2.51 ± 1.03	
		< 0.001		< 0.001		< 0.05
Postcurettage	30.00 ± 7.06		15.38 ± 4.39		2.00 ± 0.31	
Postinitial treatment	26.17 ± 8.33		11.75 ± 5.53		2.51 ± 1.03	
		< 0.001		< 0.001		< 0.01
Postflap operation	31.08 ± 5.25		17.00 ± 3.13		1.84 ± 0.16	
Postcurettage	30.00 ± 7.06		15.38 ± 4.39		2.00 ± 0.31	
		> 0.05		< 0.01		< 0.01
Postflap operation	31.08 ± 5.25		17.00 ± 3.13		1.84 ± 0.16	

Table 2. Comparation of plaque and gingival index values obtained in all phases

Treatment phase	Gingival index mean ± SD	*p*	Plaque index mean ± SD	*p*	Pocket depth (mm) mean ± SD	*p*
Pretreatment	1.14 ± 0.4		1.99 ± 0.5		2.76 ± 0.6	
		< 0.001		< 0.001		< 0.001
Postinitial treatment	0.55 ± 0.2		1.26 ± 0.5		2.36 ± 0.5	
Pretreatment	1.14 ± 0.4		1.99 ± 0.5		2.76 ± 0.6	
		< 0.001		< 0.001		< 0.001
Postcurettage	0.54 ± 0.2		1.23 ± 0.4		1.96 ± 0.4	
Pretreatment	1.14 ± 0.4		1.99 ± 0.5		2.76 ± 0.6	
		< 0.001		< 0.001		< 0.001
Postflap operation	0.53 ± 0.2		1.25 ± 0.4		1.55 ± 0.3	
Postinitial treatment	0.55 ± 0.2		1.26 ± 0.5		2.36 ± 0.5	
		> 0.05		> 0.05		< 0.001
Postcurettage	0.54 ± 0.2		1.23 ± 0.4		1.96 ± 0.4	
Postinitial treatment	0.55 ± 0.2		1.26 ± 0.5		2.36 ± 0.5	
		> 0.05		> 0.05		< 0.001
Postflap operation	0.53 ± 0.2		1.25 ± 0.4		1.55 ± 0.3	
Postcurettage	0.54 ± 0.2		1.23 ± 0.4		1.96 ± 0.4	
		> 0.05		> 0.05		< 0.01
Postflap operation	0.53 ± 0.2		1.25 ± 0.4		1.55 ± 0.3	

Table 3. Statistical evaluation of correlation between T-lymphocyte values and clinical parameters at pretreatment and post-treatment phases (initial treatment and curettage, and surgical operation)

Treatment phase	Gingival index mean ± SD	Plaque index mean ± SD
Pretreatment		
$CD4^+$	−0.092	−0.176
$CD8^+$	0.267	−0.205
$CD4^+/CD8^+$	−0.292	0.075
Postinitial treatment		
$CD4^+$	0.465*	0.347
$CD8^+$	0.360	0.475*
$CD4^+/CD8^+$	−0.073	−0.412
Postcurettage		
$CD4^+$	0.459*	0.333
$CD8^+$	0.356	0.472*
$CD4^+/CD8^+$	−0.069	−0.409
Postflap operation		
$CD4^+$	0.110	0.319
$CD8^+$	0.024	0.217
$CD4^+/CD8^+$	−0.412	−0.271

*$p < 0.05$.

The ratio of T-helper/T-suppressor cells ($CD4^+/CD8^+$) is used as an important index that determines the character of an immune response. Contradictory results have been obtained in studies on the character of the $CD4^+/CD8^+$ ratio in periodontal diseases (16–19, 28, 29).

Studies are carried out to investigate this rate, at a local and systemic level, with the purpose of determining the immunoregulation mechanisms effective in periodontal disease. There has not been a consensus yet as to the nature of $CD4^+/CD8^+$ rate in peripheric blood and periodontal disease lesions in the face of results obtained today. To this end, the studies done with gingival tissue have been carried out on tissue extracts or tissue sections. Some researchers have used the technique of cell extraction on the subjects with periodontitis and have found a $CD4^+/CD8^+$ rate lower than healthy tissue (20, 31). A few of the studies carried out on tissue sections report the $CD4^+/CD8^+$ rate in lesion to be lower than healthy tissue (13, 16–18, 21, 32), whereas some other studies report this rate to be on the increase (12, 19). On the other hand, there are some studies that maintain that there is not a significant difference in this rate in different periodontitis groups (22, 33). We, too, evaluated the tissue extracts with a flow cytometry instrument and analyzed the results to find differences in the $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ rate at treatment stages of the patients with chronic periodontitis.

In our study, we intended to remove the inflammation from the regions with chronic periodontitis, to restore healthy gingiva and to control subgingival flora through curettage and surgical operation. Okada *et al*. (34) indicated that T-lymphocyte subsets that were lower in the pretreatment phase returned to normal after well-applied curretage for all patients. Regarding our biopsy results, we determined that an increase in $CD4^+$ and $CD8^+$ continued and that results were statistically significant; the $CD4^+/CD8^+$ ratio continued to decrease, and the result was statistically significant.

Biopsy samples and clinical parameters were re-evaluated after all treatments. According to the findings, there was a negative correlation between clinical parameters and T-lymphocyte subsets. Our findings confirm the data of the earliest studies in this field (35, 36). However, the findings of Okada *et al*. (20) indicated that $CD8^+$ decreased after treatment. We suppose that some factors, such as different activities at lesions, different techniques, different treatment duration and stress during the occurrence of the diseases, may have influenced these results.

There was a decrease at scores after treatments in our study, which was found to be statistically significant. These data confirm those of the literature (1, 36–39).

In some studies, no correlation between plaque index and T-lymphocyte subsets was indicated (36–39), in other studies, a negative correlation between plaque index and both $CD4^+$ and $CD8^+$ lymphocyte is mentioned (39). In our study, a negative

relation between $CD4^+$ and $CD8^+$ lymphocyte values and plaque index after treatments were determined.

An increase in response inflammation of the host should be expected because microorganisms that cause chronic periodontitis colonize at the supra-subgingival region and bleeding occurs at gingiva in infected regions. Gingival index scores in our study decreased after treatments compared with in the beginning phase, and this result was found to be statistically significant. Our results were similar to those of previous studies (40–42).

There are studies that examined the correlation between gingival index and T-lymphocyte subsets (36–38). Although Stashenko *et al*. (36) found a negative correlation between $CD4^+$ and gingival index, some other researchers found no correlation (37, 38). Also, in our study, a negative correlation between gingival index after treatments and $CD4^+$ lymphocyte values was determined.

Consequently, these findings suggested that the local immune response was poor in the chronic periodontitis. T-lymphocytes could play a significant role in chronic periodontitis pathobiology.

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