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The Number of Inflammatory Cells in Generalized Periodontitis Versus Healthy Periodontium

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Abstract

Background: Periodontal disease is a complex chronic inflammatory condition primarily initiated by an uncontrolled inflammatory response to bacterial colonization of the tooth surface. The subsequent degeneration of periodontal tissues results from the host's inflammatory reaction to the microbial challenge. Purpose: The purpose of this research was to examine the quantitative of inflammatory cells in clinically confirmed states of periodontal health and disease based on the recent periodontal classification criteria.

Materials and methods: This cross-sectional study enrolled 88 systemically healthy individuals, divided into two groups based on their periodontal status (healthy periodontium and periodontitis). Gingival tissue samples were obtained from them. Thorough periodontal measurements, such as bleeding on probing, probing pocket depth, and clinical attachment loss, were documented. Tissue samples were fixed in paraffin and then processed to quantify the population of inflammatory cells.

Results: a significant difference (p-value < < 0.0001) was found in the scores of the inflammatory cells between periodontitis and healthy periodontium tissue samples. However, there were no significant variations in the number of inflammatory cells among different stages of periodontitis (p-value=0.528). Notably, the number of dendritic cells differed significantly between the two groups.

Conclusion: The results demonstrate that the quantity of inflammatory cells is markedly higher in periodontitis-afflicted individuals than those with healthy periodontium. Notably, the number of inflammatory cells did not show a significant correlation with the stages of periodontitis. These findings shed light on the immune response dynamics in periodontal disease and underscore the importance of understanding inflammatory cell profiles in periodontal health and disease management.

Introduction:

Periodontitis is a multicausal, complex, chronic inflammatory condition characterized by immunological dysregulation and it is influenced by different simultaneous, interrelated factors[1-4]. it is initiated by microbial colonization of the tooth surface, which triggers a cascade of inflammatory processes leading to the loss of periodontal tissue [5]. The pathogenesis of periodontitis is closely associated with the host's immune response, dysbiosis in the subgingival biofilm, and the influence of additional risk factors, which could be genetic or environmental risk factors[6]. The chronic progression of the acute host response may result in some systemic manifestations[7].

The 2017 World Workshop Classification system for periodontal and peri-implant diseases and conditions was developed to accommodate advances in biological and clinical research since the previous International Classification of Periodontal Diseases in 1999. The recent classification uses a system that classifies periodontitis based on its progression rate and severity, using criteria like staging and grading[8,9].

Previous studies have mentioned the differences in the count and in the density of lymphocyte subpopulations and plasma cells at different stages of periodontal disease. Early and stable periodontal lesions are primarily characterized by the presence of T lymphocytes, while advanced and progressive lesions exhibit a predominance of B lymphocytes and plasma cells[10-12].

A higher CD14 level has been seen in apical periodontitis, this variation in CD14 levels could be related to the innate immune response triggered by the detection of lipopolysaccharides, endotoxins, and peptidoglycan[13]. Furthermore, the role of beta-cells in the body's defence mechanism against oxidative stress has been explored, providing information about their proliferation during pregnancy and their effect on insulin sensitivity[14]. Various causes could be associated with the loss of periodontal tissues and increased probing pocket depth (PPD). These factors encompass the modified functionality of polymorphonuclear cells, alterations in host defences, and disrupted tissue homeostasis caused by a long-established microangiopathic effect[15].

Dendritic cells (DCs) are critical as immune guards and exist in a dormant state in lymphoid or peripheral organs. Within the oral environment, DCs are essential for activating and polarizing the native T cells, contributing to developing immunity and tolerance. Oral DCs, functioning as antigen-presenting cells, are widely distributed in lymphoid and non-lymphoid organs, bridging innate and adaptive immunity by triggering antigen-specific immune responses. Interestingly, oral DCs can encounter commensal bacteria and food antigens without provoking unfavorable immune reactions due to their tolerogenic nature[16]. Evidence suggests that the oral epithelial microenvironment plays a crucial role in maintaining DCs in a tolerogenic state[17].

This study aimed to quantitatively analyze the histopathological features of human gingival tissue, comparing healthy gingiva with periodontitis-afflicted tissue, and also to assess the inflammatory cells' level in the oral mucosa.

This investigation sought to evaluate the inflammatory cell profiles in clinically verified states of periodontal health and disease. By exploring the dynamics of inflammatory cell populations, this research may contribute to a better understanding of the immune response involved in periodontitis, highlighting the importance of the role of inflammatory cells in characterizing periodontal health and disease management.

Materials and Methods

Study Design and Participants:

This is a cross-sectional study that enrolled eighty-eight systemically healthy individuals who were non-smoker and older than 14 years. They sought dental care at the College of Dentistry, University of Baghdad, between May 2021 and July 2022. The protocol for the research was approved by the institution's Research Ethics Committee, and all of the procedures were carried out in accordance

with the Declaration of Helsinki in 2013. Every participant gave their consent after being fully informed about the study and signed an informed consent.

The participants were divided into two groups based on their periodontal status. The study group comprised patients diagnosed with periodontitis who required periodontal treatment using the modified Widman flap approach. Diagnosis of periodontitis was confirmed through clinical and radiographic examination of selected sites. The control group consisted of subjects who had healthy periodontium. Participants with systemic disorders, smokers, women who were pregnant or breastfeeding, individuals who had taken antibiotics or anti-inflammatory drugs within the preceding six months, and individuals who had previously received periodontal therapy were not allowed to take part in the study.

Periodontal Examinations:

The clinical and radiological evaluations of the patients was carried out by a single calibrated examiner. The oral clinical evaluations included measurements of pocket depth (PD)[18], clinical attachment level (CAL), bleeding on probing (BOP)[19], and plaque index (PI)[20]. These measurements were done by using a Williams-type periodontal probe (Hu-Friedy; Chicago, Illinois, United States of America), the measurements were obtained from six different locations surrounding each tooth. intra-examiner reliability was evaluated by, the Kappa-Cohen test, With a value of 0.90 to ensure a good level of consistency across all clinical criteria.

Periodontal Status Assessment:

According to the criteria of the World Periodontal and Peri-Implant Diseases and Conditions Classification Workshop in 2017, the participants were divided into two groups [21,22]:

The control group included 27 individuals with good periodontal health, characterized by clinically healthy gingiva[23], BOP less than 10%, PPD equal or less than 3 mm, no attachment loss, no evidence of radiographic alveolar bone loss, and should have no history of periodontitis.

The study group comprised 61 patients with generalized periodontitis. For the study group, tissue samples were obtained from sites with bone loss which were currently unstable. In contrast, for the control group, samples were collected after extraction for orthodontic reasons or from patients who underwent gingivectomy for esthetic purposes such as crown lengthening and correction of gummy smile.

Tissue Preparation and Staining:

Tissue specimens were fixed in 10% formalin and routinely processed into paraffin blocks. Sections thickness of 4µm were prepared and mounted on standard\ glass slides for hematoxylin and eosin (H&E) staining. The sections were then evaluated under a light microscope to determine the number of inflammatory cells. Additionally, 4µm thick sections were cut and mounted on positively charged slides for immunohistochemical staining using primary antibodies (anti-vimentin) to assess the number of DCs.

Inflammatory Cell Infiltration Scoring System:

The slides were captured using a microscope camera equipped with a 10-megapixel resolution (OPTIKA, Italy). The photographs were divided into 16 squares, and the number of inflammatory cells within each square was tallied to get the average count for each group. The inflammatory cells were classified into four categories based on the number of cells present: negative reaction or score 0 (0-25 inflammatory cells), mild or score 1 (26-50 inflammatory cells), moderate or score 2 (51-75 inflammatory cells), and severe or score 3 (greater than 75 inflammatory cells). as shown in figure 1.

Vimentin Scoring System:

Using a numerical scale, the intensity of cytoplasmic staining (vimentin) was assessed[24]. A negative expression was denoted by -, a weak expression by +, a moderate expression by ++, and a strong expression by +++. When each intensity level was used, the percentage of cells that

were stained was rated as follows: 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), and 4 (>75%). The intensity score and percentage of positive cells were multiplied to derive the final scores [25].

Evaluation of Staining Results:

For immunohistochemical evaluation, at least five representative fields were selected for each tissue section, examined microscopically using a 40X objective, and the mean positive percentage was recorded for each case[26].

Statistical Analysis:

GraphPad Prism version 6 for Windows (GraphPad Software, La Jolla, California, United States) was utilized to carry out the statistical analysis. When conducting descriptive statistics, it was necessary to compute percentages and the mean $\pm$ the standard deviation (SD). When analyzing the distribution of continuous variables, the Shapiro-Wilk normality test was utilized as the assessment tool. The Mann-Whitney test, Fisher's exact test, Chi-square test, and Spearman correlation test were all utilized in the process of inferential analysis using statistical methods. Statistical significance was determined to be present when the probability values were less than 0.05.

Results:

This study included 61 individuals diagnosed with periodontitis, with an average age of 44.3 + 9.8 and a median age of 44 years. Regarding the gender 22 were males (36.1%), and 39 were females (63.9%). A total of 61 tissue samples were collected for evaluation.

Conversely, the control group comprised 27 individuals with a healthy periodontium from which 27 tissue samples were obtained, with an age range of 14-45 years, a mean (SD) age of 24 (7.8) years, and a median age of 22 years. Among them, 9 were males (33.3%), and 18 were females (66.7%). The difference in age between the two groups was statistically significant, while the gender difference between the groups was not statistically significant, as shown in Table 1.

There was a significant difference in inflammatory cell scores between the study group (periodontitis) and the control group. The study group exclusively showed a negative reaction (score 0), whereas the control group had no severe reaction (score 3), as shown in Table 2.

However, there were no significant differences in the number of inflammatory cells among the different stages of periodontitis, as shown in Table 3

Furthermore, a weak positive correlation was found between the number of inflammatory cells and the probing pocket depth (PPD) ($r = 0.3$, $P = 0.028$).

Regarding dendritic cells, a significant difference was observed between the two groups. The control group showed a negative reaction (score 0) in all samples, while the study group predominantly exhibited a mild reaction, as shown in Table 4.

Discussion

The immune response to bacterial attack is known to play a significant role in the development of periodontal disease[27]. The periodontitis and healthy periodontium groups. Specifically, the periodontitis group exhibited a negative reaction (No score of 0 was found that meant the percentage of inflammatory cells was more than 5%), while the healthy periodontium group did not exhibit a severe reaction (No score 3 was found that mean the percentage of inflammatory cells was less than 51%). This aligns with the findings of Zekonis et al. in 2014 [28], who reported higher lymphocyte and macrophage counts in periodontitis patients compared to healthy individuals[29]. Based on the analysis of inflammatory cells, it was evident that the control group exhibited the lowest percentage value, while the periodontitis group demonstrated the highest percentage value. These results align with previous studies by Zekonis et al., who observed higher lymphocyte and macrophage counts in patients with chronic periodontitis compared to healthy individuals[30]. Castro et al. also reported an increase in lymphocyte numbers in gingival tissue

samples over time, correlating to the severity of chronic periodontitis[31].

Interestingly, we found no significant differences in the number of inflammatory cells among the various stages of periodontitis. Notably, severe and moderate reactions were more prevalent in stage 4 periodontitis. This lack of correlation between the number of inflammatory cells and the stage of periodontitis may be attributed to the new classification system, which primarily relies on attachment loss rather than pocket depth for staging. However, a positive correlation was observed between inflammatory cell numbers and probing pocket depth. These findings align with the research by Choi et al., who reported distinct bacterial profiles, prevalence, and inflammatory cell responses among groups with different degrees of periodontal disease[32]. Other studies have also demonstrated variations in the number and type of inflammatory cells at different stages of periodontal disease. Early and stable lesions were associated with T lymphocytes, while more advanced and progressive lesions showed a prevalence of B lymphocytes and plasma cells [10,11].

In the context of DCs identification, we employed the anti-vimentin protein immunohistochemical marker, which proved effective in labelling these cells. DCs were mainly present in the oral gingival epithelium, particularly in the basal and suprabasal layers. These findings support the findings of previous studies[33], in which it was reported about the presence of DCs scattered across the basal and suprabasal keratinocytes of the mucosal squamous epithelium. In addition, dendritic cells were found to be more intense in cases of periodontitis, showing significant variation in gingival inflammation between the studied groups. This difference in DCs between healthy and periodontitis specimens could be realted to their increased intensity in periodontitis[34]. In this study, all 61 cases of periodontitis showed positive staining for the anti-vimentin antibody, with predominantly of mild distribution of immunostained cells (score 1). while the control group showed a negative reaction (score 0), indicating fewer than 25 DCs distributed in the epithelial layer.

Since DCs are the primary antigen-presenting cells in the gingival tissue, their expression level may be highly correlated with that of the periodontitis group, reflecting their immunomodulatory activity on the intensity of the inflammatory response[35].

In summary, this study has highlighted the role of the inflammatory cell dynamics in periodontal health and disease. The significant difference in inflammatory cell scores between periodontitis and healthy periodontium shows the importance of considering immune responses in understanding periodontal disease. The distinct pattern of DCs presence and distribution in periodontitis cases underlines their potential immunomodulatory influence on the inflammatory response. Overall, these findings contribute to our comprehension of periodontal pathogenesis and could aid in developing targeted therapeutic approaches for managing periodontal disease.

Nonetheless, further investigations are warranted to delve deeper into the intricate immunological processes underlying periodontitis and explore potential therapeutic interventions. Nevertheless, due to the study's limitations, like the presence of tissue samples from individuals with ages of 14 years old in the control group only, the lack of grading, and the limited amount of tissue samples, further research is warranted to explore these relationships in more extensive and more diverse populations, considering other potential confounding factors.

Conclusions

This study revealed notable differences in the quantity of inflammatory cells and dendritic cells between the periodontitis and healthy periodontium groups. The periodontitis group exhibited a substantially higher number of inflammatory cells and DCs compared to the control group. However, it is worth noting that the number of inflammatory cells did not exhibit a significant correlation with the stages of periodontitis,

although showing a weak association with the probing pocket depth (PPD). These findings contribute to our understanding of the inflammatory response associated with periodontal disease and underscore the importance of considering inflammatory cell dynamics in periodontal health and disease characterization.

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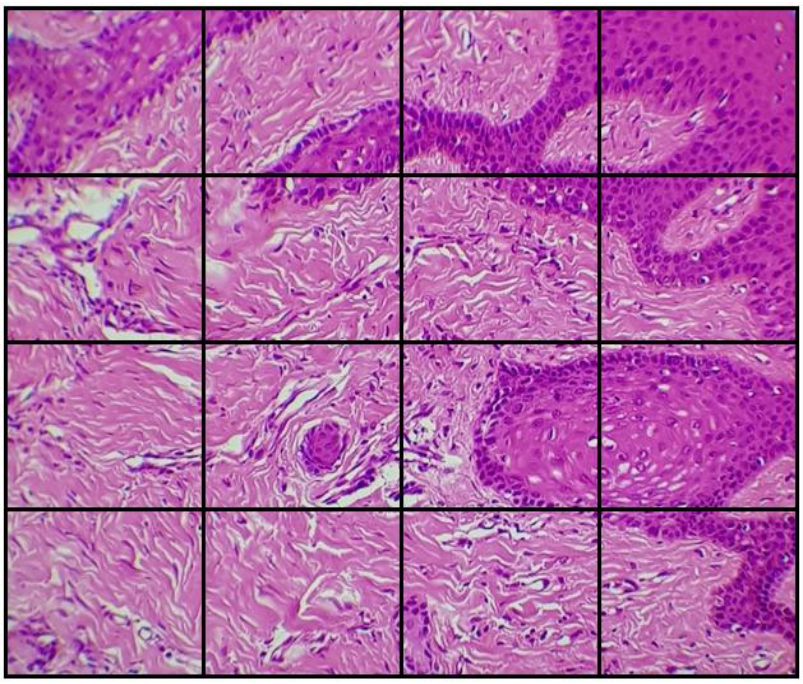

Figure (1): Designing a method for inflammatory cell counting (H&E stains).

Table 1: Statistical Analysis of Demographic Data

Age			
Group	Mean ± Sd (years)	Median (years)	P value
Control group	24 ± 7.8	22	<0.001 * Significant
Periodontitis group	44.3 ± 9.8	44	
Gender			
Group	Male N(%)	Female N(%)	P value
Control group	9 (33.3%)	18 (66.7%)	>0.05** Non-Signicant
Periodontitis group	22 (36.1%)	39 (63.9%)	

* Significant at $p < 0.05$ using the T- test, %: Percentage, N: total number, ** non-significant at $p > 0.05$ using the Chi-square test

Table 2: Difference in the number of inflammatory cells between the two groups.

Score	Number of inflammatory cells (%)		P value
	periodontitis group	Control group	
Negative (score 0)	0 (0)	12 (44.4)	< 0.0001 *
Mild (score 1)	10 (16.4)	13 (48.1)	
Moderate (score 2)	23 (37.7)	2 (7.5)	
Severe (score 3)	28 (45.9)	0 (0)	
Total	61	27	

* Significant at $p < 0.05$ using the Chi-square test. %: Percentage

Table 3: The number of inflammatory cells in different stages of the periodontitis group.

Stage of periodontitis	Number of inflammatory cells (%)			P value
	Mild	Moderate	Severe	
2	1 (1.7)	4 (6.6)	1 (1.7)	0.528 *
3	5 (8.2)	8 (13.1)	12 (19.6)	
4	4 (6.6)	11 (18)	15 (24.5)	
Total	10 (16.5)	23 (37.7)	28 (45.8)	

* Significant at $p < 0.05$ using the Chi-square test. %: Percentage

Table 4: Difference in the number of dendritic cells between the two groups.

Score	Number of inflammatory cells (%)		P value
	periodontitis group	Control group	
Negative	22 (36.1)	27 (100)	< 0.0001 *
Mild	35 (57.4)	0 (0)	
Moderate	2 (6.5)	0 (0)	
Severe	0 (0)	0 (0)	
Total	61 (100)	27 (100)	

* Significant at $p < 0.05$ using the Chi-square test. %: Percentage

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