

## Level of Calcitonin Gene-Related Peptides in Elderly and Adult Periodontitis Patients

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### Abstract

Periodontitis is an inflammatory disease caused by bacteria that forms a dental biofilm. Calcitonin gene-related peptide (CGRP) is an anti-inflammatory agent that inhibits osteoclast activity. This study compared the CGRP levels in the gingival crevicular fluid (GCF) from adult and elderly periodontitis patients. Clinical samples were obtained from the GCF of 70 subjects with periodontal diseases, including male and female subjects 60–75 years old ( $n = 42$ ) and control group adult patients 25–55 years old ( $n = 28$ ). Measurements of clinical parameters, including the probing pocket depth (PPD) and bleeding on probing, were assessed as diagnostic criteria. A pocket depth was defined as being present if the PPD was  $\geq 3$  mm. An enzyme-linked immunosorbent assay was performed to measure the CGRP levels. The level of CGRP in the GCF was slightly higher in adult patients (39.91 pg/ $\mu$ g) than in elderly patients (25.52 pg/ $\mu$ g); however, this result was not statistically significant ( $p > .05$ ). The level of CGRP in the GCF of periodontitis patients was not dependent on age and was lower in elderly patients.

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### Introduction

Periodontitis is an inflammatory disease that occurs chronically and is characterized by periodontal tissue damage. There is a high prevalence of periodontal disease in Indonesia. Based on the Indonesia Health Profile in 2018, the average number of periodontitis patients reached 74.1%.<sup>1</sup> The health of periodontal tissue is affected by the balance of the immune system, including immune cells and inflammation, with the ecology of bacteria.<sup>2</sup>

Inflammation is typically a protective measure to maintain and repair tissues; however, if the causative factor is persistent, it can create prolonged inflammation that can damage tissues.<sup>2,3</sup> The body has a mechanism for regulating the inflammatory response, one of which is regulated by the nervous system via neuropeptides.<sup>4</sup> Neuropeptides are released by

neurons and have various biological roles.<sup>5</sup> Calcitonin gene-related peptide (CGRP) is a neuropeptide that plays a role in the inflammatory process of periodontal tissue and is a potent vasodilator.<sup>6</sup> CGRP can increase the production of cytokines through T cells and increases the accumulation of neutrophils through interleukin-1. CGRP can also reduce inflammatory responses, such as reducing interleukin-2 production, preventing bone resorption, and stimulating bone formation.<sup>7</sup>

The older a person is, the higher the risk of infection, neoplasm, and autoimmune disorders. The rates of attachment loss are also higher in elderly patients.<sup>8</sup> This is due to a dysregulation of the immune system that occurs with age. These changes can affect the innate and adaptive immune system.<sup>9</sup>

To date, data on the levels of CGRP in the gingival crevicular fluid (GCF) in Indonesian elderly periodontitis patients have not been published. CGRP can be used as a biomarker to detect potential progressions of periodontitis and an indicator of the severity of periodontitis. Therefore, we evaluated the CGRP levels in adult and elderly periodontitis patients.

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## Materials and methods

This study was conducted at the Elderly Social Institution in Jakarta, Indonesia, from May to July 2019. The laboratory work was done at Indonesia Medical Education and Research Institute (Jakarta). The Ethics Committee at the Faculty of Dentistry, University of Indonesia, approved the study protocol. The clinical exam was performed, and samples were collected at the Universitas of Indonesia by a group of periodontology residents.

A total of 78 patients participated in this cross-sectional study, 28 men (37%) and 50 women (65%). There were 42 elderly patients aged 60–75 in the test group and 28 adults aged 25–55 in the control group. All of the subjects signed an informed consent letter to participate in this study. The inclusion criteria were periodontitis patients with a pocket depth of 3–4 mm with no gingival recession. Periodontitis patients were excluded if they had a systemic disease that could affect the periodontium, such as diabetes mellitus, history of smoking, currently pregnant, or recently received periodontal therapy. An intraoral examination was performed by measuring the pocket depth, papilla bleeding index, plaque index, calculus index, and oral hygiene index using Periodontal Probe UNC 15 (Osung Mnd Co., Ltd., Korea).

After the examination, a sample of the GCF was collected. The GCF was obtained from one random site with a pocket depth of 3–4 mm. First, the site was cleaned to remove supragingival plaque. Next, the tooth was dried using cotton pellets. The GCF was collected by isolating the site with cotton rolls and gently inserting paper points #20 (Dentsply, North Carolina, USA) into the pockets for 30 seconds. The GCF from each tooth was collected using three paper points and stored in an Eppendorf tube filled with phosphate buffer saline. Samples were stored at -80°C until an analysis was performed using an ELISA kit (Elabscience, Houston, TX, USA). A Mann–Whitney test was performed using SPSS software (SPSS, Chicago, IL, USA).

## Results

The demographic data and periodontal parameters are shown in Table 1. The CGRP levels in the GCF between the test (elderly

periodontitis patients) and control groups (adult periodontitis patients) were not statistically significant as shown in Table 2 with  $p$  value 0.47 ( $p > .05$ ).

Parameter	Test group	Control group
	Mean [SD]	Mean [SD]
Age	66.34 [5.64]	35.53 [5.61]
Periodontal parameter		
PD		
PI		
CI	2.29 [0.5]	1.99 [0.34]
OHIS	1.56 [0.75]	0.97 [0.51]
PBI	1.56 [0.91]	1.21 [0.62]
	3.16 [1.45]	2.21 [1.08]
	1.02 [0.89]	1.17 [0.76]

**Table 1.** Patient demographic and periodontal clinical parameters.

PD, pocket depth; PI, plaque index; CI, calculus index; OHIS, Oral Hygiene Index Simplified; PBI, Papilla Bleeding Index.

In general, the periodontal clinical parameter score of the test group was higher than the control group. The level of CGRP was higher in the control group ( $39.91 \pm 68.34$  pg/ $\mu$ g) compared with the test group ( $25.52 \pm 29.9$  pg/ $\mu$ g). There were notable differences in the maximum level of CGRP in the test and control groups. The maximum level of CGRP from the test group was 123.41 pg/ $\mu$ g and that from the control group was 328.34 pg/ $\mu$ g.

Parameter	Mean [SD]	Median [min–max]	$p$ value
Elderly patients (n = 42)	25.52 [29.9]	14.85 [2.20–123.41]	.47
Adult patients (n = 28)	39.91 [68.34]	11.13 [2.37–328.34]	

**Table 2.** Calcitonin gene-related peptide levels in the gingival crevicular fluid (GCF) of adult (control group) and elderly periodontitis patients (test group).

Mann–Whitney test; \* $p < .05$  indicates a significant difference.

## Discussion

Research on the CGRP levels in periodontal disease has not been widely performed, and data regarding the relationship between age and CGRP levels is limited. In this study, periodontitis patients were divided into two groups according to their age, adults aged 25–55 and elderly patients aged 55–75. The level of CGRP was measured and compared between these groups. In this study, we investigated whether the CGRP levels in periodontitis patients can be affected by age.

CGRP is a neuropeptide that plays a role in the inflammatory process and in periodontal disease. CGRP is a potent vasodilator that can act as an anti-inflammatory. CGRP also plays a role in bone remodeling, increases osteoblasts production, and decreases osteoclasts. Therefore, CGRP can be a protective factor to prevent or slow the progression of periodontitis.<sup>10,11</sup>

In this study, age did not affect the level of CGRP found in the GCF of periodontitis patients. However, the level of CGRP in the elderly group was not as high as in the younger group. According to research conducted by Jung et al., there was a decrease in the density of nerve fibers in periodontal tissues that produce CGRP in older rats.<sup>12</sup> Another study by Chai et al. stated that the production of CGRP was attenuated with the advancement of age caused by the degradation of sensory nerve fibers.<sup>13</sup>

The CGRP levels in the GCF vary depending on the severity of the periodontal disease, as shown in the study by Lundy et al. In this study, the highest levels of CGRP were found in healthy periodontal tissues, and the levels decreased as the disease progressed.<sup>10</sup> This finding was also stated in a study conducted by Yan et al. in which CGRP levels decreased in severe periodontitis, and after treatment, the level of CGRP had increased.<sup>14</sup> According to Lundy et al., this is most likely due to the presence of carboxypeptidase, which can degrade CGRP in severe periodontal disease; thereby, CGRP levels in both groups dramatically decline in the case of increasing severe damage.<sup>15</sup> CGRP can also inhibit bone resorption, as shown in an animal study by Akopian et al.<sup>16</sup>

There were some factors and limitations that could have affected the results of this study. For example, each patient had a different level and duration of inflammation, and CGRP is strongly influenced by the degree of severity; therefore, this result can be biased. CGRP is a biomarker that can predict the progression of periodontitis and help determine treatment plans for periodontitis patients. Higher levels of CGRP can be found in healthy patients, as CGRP is a protective factor. Therefore, patients with a higher level of CGRP will have a lower risk of periodontitis. On the other hand, lower CGRP levels can be a sign of ongoing disease and a high risk of periodontitis.

## Conclusions

The level of CGRP in the GCF of periodontitis patients was slightly lower in elderly patients than in adult patients. However, the result was not statistically significant; thus the level of CGRP was not related to age.

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## Declaration of Interest

The authors report no conflict of interest.

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