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Avidity of serum immunoglobulin G antibodies to *Porphyromonas gingivalis* in the elderly with chronic periodontitis

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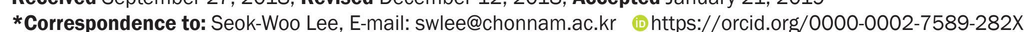
The present study aimed at evaluating serum immunoglobulin G (IgG) avidity to *Porphyromonas gingivalis* in elderly patients with mild and severe chronic periodontitis. The avidity of antibodies against *P. gingivalis* present in the sera of 18 patients with mild chronic periodontitis and 18 patients with severe chronic periodontitis was evaluated using an ammonium thiocyanate-dissociated enzyme-linked immunosorbent assay (ELISA). The results showed that the mean absorbance value in serum IgG antibody titers was significantly higher in the severe chronic periodontitis group than in the mild chronic periodontitis group (198 ± 35 ELISA unit [EU] vs. 142 ± 32 EU, $p < 0.01$). However, there was no significant difference between the two groups in antibody avidity (65 ± 57 EU vs. 54 ± 27 EU). These findings suggest that humoral immune responses to *P. gingivalis* between mild and severe chronic periodontitis in elderly patients are characterized by the differences in the quantity rather than the quality of the antibodies.

Keywords: *Porphyromonas gingivalis*, Immunoglobulin G, Antibody affinity, Chronic periodontitis, Geriatrics

Introduction

Periodontal disease is a common infectious disease that can lead to a destruction of the supporting structures of tooth and, ultimately tooth loss, if not treated [1,2]. It has been determined that dental plaque biofilm, consisting of more than 700 different bacterial species and their products, is an etiological agent of periodontal disease [3,4]. Immunological and inflammatory responses by the host to dental plaque biofilm via host-parasite interaction are manifested by signs and symptoms of periodontal disease [2,5]. There are many risk factors that can affect host-parasite interaction in periodontal disease, modifying influence on the host susceptibility and the progression of periodontal disease [2].

It has been suggested that the immune function in periodontal disease is a “double-edged” sword, fighting pathogens and eliciting tissue damage in the host, as both cell-mediated and humoral immune responses against periodontal pathogens are well documented in the periodontal lesion [6,7]. In fighting periodontal infections, pathogen-specific antibodies may be effective in reducing or inhibiting bacterial colonization, proliferation, and spread. It was observed that antibody titers vary greatly between patients and also before and after therapy [8,9]. In some cases, antibody titers may reflect a host’s previ-



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ous exposure to periodontal pathogens, as a high titer could be considered as a positive immune response by the patient, but could also reflect an inability of the immune system to remove the pathogen [10]. Elevated serum immunoglobulin G (IgG) antibody to periodontal pathogens may indicate destructive periodontal disease status, and may be considered as a risk factor for disease progression [11,12]. On the other hand, numerous clinical and animal studies have shown that serum antibody titers do not always correlate with the clinical stages of periodontitis [7].

Typically, the humoral immune responses to an infectious agent are expressed in terms of antibody titers without considering the quality of the antibody that is expressed in terms of avidity. Antibody avidity is a measure of the net binding strength between multivalent antigen and polyclonal antibodies, reflecting the functional activity of the antibodies [13]. It is generally accepted that a variety of immune functions, including complement activation, opsonization, and toxin neutralization are more effective with high-avidity antibodies [13,14]. Therefore, avidity expressed as "functional affinity", is measured as adequate assessment of the strength of the antibody in binding and eliminating antigens.

While humoral immune responses to *Porphyromonas gingivalis* have been examined in numerous studies for disease progression [11,15,16] and treatment effects [17,18], there have been few, if any, reports on the quality of these antibodies. There are inconsistent reports on the antibody avidity to *P. gingivalis*. Lopatin et al. [15,16] found elevated IgG antibody avidity to *P. gingivalis* in chronic periodontitis patients aged 23 to 51, compared with healthy controls. A study by Holbrook et al. [17] noted higher antibody avidity to *P. gingivalis* in refractory periodontitis, which did not change with therapy. On the other hand, Chen et al. [18] reported that generalized aggressive periodontitis patients (mean age: 33.5 years) had lower antibody avidity, which increased following non-surgical therapy. In addition, it appears that periodontal therapies result in a decrease in antibody titers and an increase in the avidities to *P. gingivalis* [19-21], although a few studies reported a reduced antibody avidity to *P. gingivalis* after therapy [8]. However, these studies have observed the antibodies avidities among adult patients aged under 50. Therefore, very little information has been available regarding the avidity of antibodies to periodontal pathogens in the geriatric patients with chronic periodontitis.

Considering the possibility that efficiency of antibodies against periodontal pathogens may influence the progression of periodontal disease in the elderly, new information regarding the functional activity of antibodies is required to better understand the natural history of chronic periodontitis in the elderly patients. The purpose of this study was therefore to evaluate the avidity of serum IgG against *P. gingivalis* in geriatric patients with chronic periodontitis.

Materials and Methods

1. Subjects

The protocol of this study was approved by the Institutional Review Board of the Chonnam National University Hospital, Gwangju, Korea (IRB no. #1-2008-05-226). This study population included 85 geriatric subjects (37 males and 48 females), 60 to 82 years old, who were residents of Gwangju and recruited for a public health survey performed jointly by Chonnam National University Medical School and School of Dentistry, Gwangju, Korea. Subjects who were previously exposed to antibiotics in the past four months and having chronic inflammatory and immune disorders were excluded in this survey. Each subject received a standard periodontal examination by a periodontist and was assigned to one of three groups based upon the severity of periodontitis according to the criteria proposed before [22]. These groups are divided as follows: (1) mild chronic periodontitis (MCP; N = 29, mean age: 64.1 years, range: 60 to 70 years) group with clinical attachment loss (CAL) less than 3 mm, (2) moderate chronic periodontitis (MoCP; N = 27, mean age: 67.6 years, range: 60.8 to 83 years) group with CAL of 3 to 5 mm, and (3) severe chronic periodontitis (SCP; N = 29, mean age: 68.5 years, range: 60.9 to 82 years) group with CAL of more than 5 mm.

2. Serum collection

Serum samples were provided by the Department of Pathology, Chonnam National University Medical School, Gwangju, Korea. Serum samples were collected from chronic periodontitis patients during a public health survey performed under the protocol (#1-2008-05-226). Blood was drawn by antecubital vein puncture and was allowed to coagulation for 30 minutes at 37°C. Serum was separated by centrifugation at 2,000 *g* for 10 minutes at 4°C, then aliquoted in 1 mL and stored at -20°C until use.

3. Growth of *Porphyromonas gingivalis*

P. gingivalis 381 was grown in anaerobic chamber (85% N_2, 10% H_2, 5% CO_2). Tryptic soy broth 4% (w/v) supplemented with yeast extract 0.5% (w/v), cysteine 0.05 (w/v), 10 mg/L hemin, 5 mg/L which was autoclaved at 121℃ for 15 minutes was used for bacterial culture.

4. Protein extraction from bacterial cells

SMART™ bacterial protein extraction solution (iNtRON Bio-technology, Seongnam, Korea) was used to prepare the bacterial cell lysate. Briefly, bacterial cells were grown and adjusted to concentration at OD_{600} = 1.5–3.0, and these were washed 3× with phosphate buffer saline (PBS), and subsequently harvested at 13,000 rpm for 5 minutes at 4°C. Bacterial cell pellets were vortexed vigorously and resuspended in 350 μL of SMART™ solution. These solutions were subjected to centrifugation at 13,000 rpm for 5 minutes in 4°C to separate the insoluble protein from the soluble. The supernatant containing the soluble protein was transferred to a clean tube. A QuantiPro BCA assay Kit (Sigma-Aldrich, St. Louis, MO, USA) was used for measuring the protein concentration.

5. Measurement of serum immunoglobulin G antibody titers

P. gingivalis cell lysates were added to each well (100 ng/well) in a polystyrene microtiter plate, and the plates were incubated overnight at room temperature. The plates were then washed 2× with Tris buffered saline with 2% Tween (TBS-T), and 200 μL of 2% skim milk solution was added and incubated for one hour at 37°C. After the wash with TBS-T, patient's sera (1 : 100 dilutions) were added to the plates, and mixed for 2 hours at 37°C with a gentle agitation. Then, the plates were washed with TBS-T 3× , and rabbit anti-human IgG conjugated with horse radish peroxidase (1 : 10,000 dilutions) (Abcam, Cambridge, UK) was added. The plates were incubated for one hour at 37°C then washed 5× with TBS-T. Subsequently, 50 μL of TMB substrate solution (GenDEPOT, Katy, TX, USA) was added for the color development, and the reaction was stopped after 2 minutes by adding 100 μL of the stop solution, followed by OD reading at 450 nm by Versamax ELISA Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). All experiments were performed in triplicates.

The mean absorbance values from the triplicates were used for analysis. Relative antibody levels were expressed as enzyme-linked immunosorbent assay units (EUs) measured from a standard curve created by assigning a value of 100 EU to a single sample of patient serum. Sample antibody levels were determined from the absorbance value plotted on the standard curve and expressed as EU.

6. Measurement of serum immunoglobulin G avidity levels by dissociation analysis

Relative levels of IgG avidity were determined by measuring the dissociation of antibody-antigen binding caused by ammonium thiocyanate as described before [16] with a slight modification. The microtiter plates were sensitized with antigen as described above. After washing with TBS-T, a single dilution (1 : 100) of each sera was added to each well in the plate. After incubation for one hour at 37°C, the plates were washed with TBS-T, and 0, 1, and 2 M (1.0 mL/well) of ammonium thiocyanate were added to each well. After an incubation for one hour and three washes with PBS-T, the assay proceeded as described above. Percent antibody binding ([Absorbance of thiocyanate treated well/absorbance of control well] × 100%) was calculated for each concentration of thiocyanate ion used. The concentration of thiocyanate ion required to inhibit 50% of the bound antibody was calculated by linear regression analysis as described before [16].

7. Statistical analysis

All experiments were performed in triplicates to ensure data accuracy and the average of the triplicate data was used for the analysis. All statistical analyses were performed by using statistical software IBM SPSS Statistics 19.0 (IBM Corp., Armonk, NY, USA). Student's t-test was used to evaluate the significance of differences in the antibody titers and avidity values when mild and severe disease group were compared. The relationships between antibody titer and avidity in these groups were tested using regression analysis. An alpha of 0.05 was used as the cut off for statistical significance.

Results

1. Serum immunoglobulin G titers and relative avidities

Eighteen serum samples from patients with MCP were com-

pared to an additional 18 serum samples from subjects having SCP. As shown in Table 1, the mean IgG titers of the SCP group were significantly elevated when compared to those of the MCP group (198 ± 35 EU vs. 142 ± 32 EU; $p < 0.01$). However, when the avidity of anti-*P. gingivalis* antibodies of the IgG in the sera of SCP and MCP patients were evaluated, there was no significant difference between the two groups (65 ± 57 EU vs. 54 ± 27 EU).

2. Relationship between titer and avidity

A regression analysis was performed to determine if there was a significant relationship between the levels of antibody titer and avidity. It was found that no significant relationships existed between titer and avidity both in the SCP and MCP groups ($r^2 = 0.04$, severe; $r^2 = 0.003$, mild).

Discussion

Previous studies have suggested that older adults with chronic periodontitis exhibit humoral immune responses to *P. gingivalis* as efficient as the younger adults [23–28]. However, most of these studies assessed only the quantity of IgG, not the quality. The purpose of this study was therefore to evaluate the avidity of serum IgG against *P. gingivalis* in geriatric patients with chronic periodontitis, and compare the levels based on the severity of disease.

Confirming other studies, the present study found that the mean IgG titer is significantly elevated in the sera of patients having severe periodontitis, compared to those with moderate periodontitis. The higher antibody titers were not unexpected since it probably represented a more vigorous, repeated antigenic challenge with *P. gingivalis* in those subjects with SCP, compared to MoCP [12].

However, evaluation of the avidities of the IgG antibodies did not exhibit a significant difference between SCP and MoCP. These findings suggest that the quality of antibody to *P. gingivalis* does not affect the severity of chronic periodontitis in geriatric patients.

Table 1. Antibody titer and avidity of mild and severe chronic periodontitis patient serum immunoglobulin G antibodies reactive with *Porphyromonas gingivalis*

Periodontitis	Mild	Severe	Significance[a]
Titers (EU ± SD)	142 ± 32	198 ± 35	$p < 0.01$
Relative avidity (ID_{50} ± SD)	54 ± 27	65 ± 57	Not significant

EU, enzyme-linked immunosorbent assay unit; SD, standard deviation.
[a]Student's t-test.

There is no consensus in predicting the exact roles of avidity in assessing the severity and progression of chronic periodontitis. It was found that chronic exposure to antigens can lead to low avidity despite the presence of high antibody titers [29], resulting in the reduced protective function [30]. It was reported that IgG avidity to *P. gingivalis* in patients with aggressive periodontitis was lower than that of healthy subjects [31,32], and the avidity increased after the periodontal treatment [21], suggesting that the protective role of IgG avidity in periodontal infection. On the other hand, it was demonstrated that IgG avidity in chronic periodontitis patients was higher than that of healthy subjects [15,16]. These conflicting results indicated that IgG avidity may not be a predictable indicator for the status and/or progression of chronic periodontitis. Instead, antibody titers to *P. gingivalis* may be more useful as a disease marker [28].

This study has several limitations. Serum samples were selected only from geriatric patients having MCP or SCP, not including control subjects without periodontitis. This study was performed as a parallel study using the same geriatric patients examined in our previous studies [28,33], in which the purpose was to assess the levels of periodontal pathogens and characterize humoral immune responses on the basis of the severity of chronic periodontitis. It would be very difficult to obtain periodontally healthy cohorts from the geriatric subjects, since most elder patients are presumed to have some degree of chronic periodontitis. Therefore, the control group without chronic periodontitis was not included in the present study. In addition, in this study patient samples were analyzed only for the cases of MCP and SCP excluding those of MoCP, since the previous results showed that there was no significant differences in the IgG titers between groups of MoCP and SCP, while showing a difference between groups of MCP and SCP [28]. Therefore, only the mild and severe periodontitis groups were compared in the present study.

Despite several evidences suggesting a link between antibody titers or avidity and the severity of chronic periodontitis, the exact mechanisms involving the association have not been fully elucidated. Therefore, well-designed future studies or large scale big data analyses are required to clarify the biological mechanisms and clinical relationships between the antibody quality and the status of chronic periodontitis.

In conclusion, these findings suggest that the differences in humoral immune responses to *P. gingivalis* between MCP and

SCP in elderly patients are characterized by the difference in the quantity, rather than the quality of IgG antibodies.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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