

## Research Report

## Immunoglobulin-G level on aggressive periodontitis patients treated with clindamycin

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### ABSTRACT

**Background:** Aggressive periodontitis might occur as a result of complex interplay between bacteria and host defence, therefore, the host susceptibility plays important role. Antimicrobial agents that could enhance host defence are required. Clindamycin might influence host defence. **Purpose:** The purpose of this study was to determine the influence of clindamycin on level of Immunoglobulin-G (IgG) patients with aggressive periodontitis, and its mechanism. **Methods:** This study used the pre-test post-test control group design. Eighteen aggressive periodontitis patients were divided into 2 groups at random. Group 1 (treatment): 9 aggressive periodontitis patients were given with clindamycin of 150mg orally, 4 times a day, for 7 days. Group 2 (control): 9 aggressive periodontitis patients were given with tetracycline of 250mg orally, 4 times a day for 12 days, and then metronidazole of 200mg orally, 3 times a day for 10 days. Blood sample was collected from vena cubiti mediana. Level of IgG was measured at base line and day 28. Data were analyzed statistically by using *t*-test ( $\alpha = 0.05$ ). **Result:** Examination for IgG level showed there was significant difference between pre-test and post-test ( $p < 0.05$ ). Level of IgG was significantly increased after therapy, both in treatment and control group. The increase of IgG level in treatment group was not different significantly from control group ( $p > 0.05$ ). **Conclusions:** This study shows that clindamycin can be used as drug of choice for the treatment of aggressive periodontitis since clindamycin can improve the immunity status of aggressive periodontitis patients.

**Key words:** immunoglobulin-G, aggressive periodontitis, clindamycin

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### INTRODUCTION

The treatment of periodontal diseases still focused on local factors, the quality of hosts are still less concerned. Therapy using antibiotic is usually concerned with old paradigm which aims to eliminate the bacteria. In fact, antibiotic certainly affects the immune response. Some studies show that antibiotic is immunomodulatory because it has several characteristics; micro-organism eradicator, phagocytic, chemotactic and lymphocyte activity (effect by non-antibiotic of antibiotic).<sup>1-3</sup> These characteristics of antibiotic are required to support the success of periodontal diseases treatment, specially for aggressive periodontitis.

Aggressive periodontitis is typical and it attacks the patients under 35 years old. Its clinical symptoms are good oral hygiene, less accumulation of plaque and

calculus. In contrast, there is a rapid loss of alveolar bone. Besides, much loss of attachment rapidly occurs, and it is followed by the occurrence of pocket deeper than 5mm. The amount of plaque and calculus cannot be compared with the seriousness of disease. This disease appears because there is disturbance of the immune system,<sup>4</sup> so in its treatment the efforts to increase the immunity are required. Therefore, proper strategy of treatment needs to be seriously considered. This strategy can be applied by giving medicines effectively and efficiently which aims to eliminate the bacteria and increase the immune system. Another alternative solution is by using the immunological effect of antibiotic.

Systemic antibiotic should be used in aggressive periodontitis treatment, because the micro-organisms which causing this disease is capable of invading the

pocket and leaving into sub epithelial gingival connective tissues. Clindamycin is one of antibiotics used in aggressive periodontitis. It is effective against *Porphyromonas gingivalis* and *Prevotella intermedia* which are found in pocket of aggressive periodontitis.<sup>5,6</sup> Besides as antibiotic clindamycin influences the immune response of patients.<sup>7</sup> By giving clindamycin to aggressive periodontitis patients, it is expected that the clindamycin can reduce pathogens owned by bacteria and increase the immunity. However, this statement needs further evidence and explanation.

In such a chronic disease like aggressive periodontitis, the dominant immunoglobulin is IgG.<sup>8</sup> Therefore, it is used as indicator in this study. The objective of the study was to identify the influence of giving clindamycin on IgG level of aggressive periodontitis patients. The finding of the study is expected to be used as basis in determining proper strategy of treatment so that it can increase the success of treatment.

Clindamycin is a derivate of lincomycin antibiotic which is derived from fungus named *Streptomyces lincolnensis*. It is bacteriostatic because it inhibits the protein blending of bacteria at sub unit ribosom 50S which functions as recipient. The clindamycin is tied at macrophage and leucocyte polymorphonuclear so it can increase the process of phagocytosis and intracellular killing.<sup>9,10</sup> Clindamycin is effective against anaerob bacteria either in positive gram or negative gram. It can be given per oral, intravenous and topical. Dosage per oral is 150mg–3g/day for adult, every 6 hours. For children, it is 10–20mg/kg/weight/day. Concentration in serum is 2–3µg/ml that is obtained after one hour of intake.<sup>11</sup>

Van Winkelhoff *et al.*<sup>12</sup> stated that in his study on refractory and adult periodontitis patients who were given clindamycin 150mg 4 times a day for 7 days, after a twenty-four month observation showed that there was activity decrease of disease from 10% to 0.5%, lengthening the emergence from 5 to 17 months. This study also concludes that the given dosage can inhibit the growth of bacteria like *Porphyromonas gingivalis*, *Prevotella intermedia* and *Peptostreptococcus micros* for 12 months.

Clindamycin is able to enhance the production of immunoglobulin-G in malignancy patients of epitel tumour with secondary infection. Who studies the change of imunoglobulin-G level after being given clindamycin for 12 days on animals affected by paracite which is called *Babesia gibsoni*. The disease characterized by cellular and humoral immune suppression, with levels of IgG antibody decline less rapidly in persistently infected patients. These results suggested that clindamycin damage paracites morphologically. clindamycin does not only destroy the paracite causing infection, but it also increases humoral and cellular immunity so that it can improve the clinical conditions.<sup>13–16</sup>

At first, the occurrence of periodontitis caused by disturbance of local factors. One of the factors is the presence of bacteria. Recently some researchers have believed that the growth of aggressive periodontitis

depends on the interaction between periodontopathogen with immune response. Aggressive periodontitis may occurs because there is disturbance of immune system. Aggressive periodontitis is closely related to the quality and the susceptibility of the host, caused by abnormal immunocompetent cells that have disturbance of immune response. As consequence, the susceptibility of patients towards aggressive periodontitis is increased. One of abnormalities is phagocyte cells (neutrophils and monocytes).<sup>17–21</sup>

In aggressive periodontal treatment, the antibiotics which have clinically been studied and examined for the effectiveness are tetracycline and metronidazole on 30 aggressive periodontitis patients, which are divided into two groups of treatment, one group of control given therapy with two kinds of antibiotics deliberately, concluded that tetracycline which is given to patients 250 mg 4 × a day for 12 days and metronidazole 200mg 3 × a day for 10 days give optimal treatment on aggressive periodontitis patients with clinical indicators; bleeding on probing (BOP), probing depth (PD), gingival index (GI) and suppuration.<sup>22</sup>

The purpose of this study was to determine the influence of clindamycin on level of Immunoglobulin-G (IgG) patients with aggressive periodontitis, and its mechanism. The advantage of this study was to optimize aggressive periodontitis treatment.

## MATERIALS AND METHODS

This study was designed and based on true experimental by using the post-test pre-test control group design. The population of this study was aggressive periodontitis patients who come to Periodontic Clinic at Faculty of Dentistry, Airlangga University. The criteria of the samples were as the following: male patients who are less than 35 years old; having clinically diagnosed of aggressive periodontitis, having no other infections either in oral cavity or other parts of the body, non-smokers, having no recent antibiotic therapy or other medicine.

The samples of this study, moreover, were taken by using simple random sampling technique, which was divided into two groups; treatment group and control group. Based on the trial method, each group on this study consisted of 9 the samples.

First, the blood of the patients was taken from vena cubiti mediana of their arms for about 4 cc. Then, the blood collected was put into venoject plain tubes for IgG examination. Afterwards, each patient was treated by scaling and root planing methods. For the treatment group, the patients were asked to take clindamycin of 150 mgs orally four times a day for seven days. Meanwhile, for the control group, the patients were treated with active control method by asking them to take standard medicines for aggressive periodontitis orally, which were tetracycline and metronidazole. This treatment was then divided into two stages. In the first stage, the patients were asked to

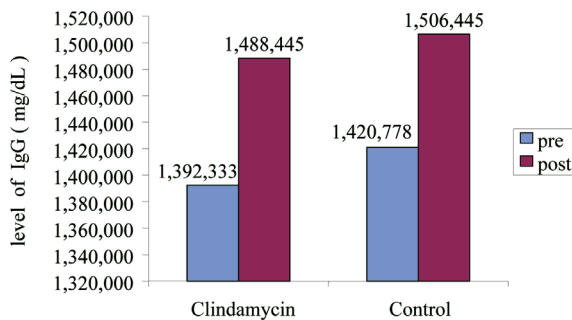
take tetracycline of 250 mgs orally 4 times a day for 12 days. And, in the second stage, the patients were asked to take metronidazole of 200mgs orally 3 times a day for 10 days.<sup>22</sup>

The IgG level, furthermore, was measured on the twenty eighth day. The reason was because the period of IgG formation took 28 days. Thus, the blood of all patients either from the group of control or from the group of treatment was taken for about 4cc, which was then put into venoject plain tubes. Afterwards, the tubes were examined based on standard laboratorial procedures for examining IgG level. The method which was used, moreover, is “turbidimetry” with “immunoglobulin turbiquant automatic analysis tool. Therefore, the blood sample in venoject plain tubes was stirred with centrifuge tool at a speed of 3000 rpm. 50 µl of serum was then taken and added with 1000 µl of NaCl isotonic liquid. Afterwards, 20 µl was taken and added with 500 µl of immunoglobulin reagent. The solution then was put into inhalator tubes which were set on “immunoglobulin turbiquant” tool. The results of the examinations finally could be read at the monitor screen.

The result data were analyzed statistically by student’s t-test ( $\alpha = 0.05$ ). However, before being analyzed, all the data had to be examined first for their normality test by using “one-sample Kolmogorov-Smirnov test” with the level of significance at  $p > 0.05$ .

**RESULT**

The level of IgG in treatment group (clindamycin) and in the control group (tetracycline + metronidazole) are shown in Table 1 and Figure 1. Kolmogorof -Smirnov test shows  $p > 0.05$  which mean the data have normal distribution, so the data can be examined by student’s t-test.



**Figure 1.** Level of IgG pre and post therapy on the treatment and the control group.

In order to analyze the difference of IgG levels in pre-test and in post-test, either in the treatment group or in the control group, the data are examined statistically by paired t-test. The result showed that  $p = 0.000$  ( $p < 0.05$ ), which means that there was a significant difference. In other words, it indicated that giving either clindamycin or tetracycline+metronidazole could significantly increase the level of IgG.

In order to analyze the differences of pre-test data among both of groups, the data were examined by independent t-test. The results showed that  $p = 0.374$  ( $p > 0.05$ ), which means that there was no significant differences among them. In other words, it indicated that the level of IgG in pre-test, either in the treatment group or in the control group, was considered the same. Therefore, in order to determine whether there was a significant difference among the groups or not, independent t-test of the post-test data of both groups were done. The result shows that  $p = 0.576$  ( $p > 0.05$ ), which means there was no significant difference. In other words, it indicated that the level of IgG in post-test, either in the treatment group or in the control group, is statistically not different. It meant that the change of IgG level in the treatment group was the same as in the control group. Therefore, the IgG level of aggressive periodontitis patients who follow the therapy with clindamycin was the same as tetracycline + metronidazole.

It might be suggested that the pattern of the change in both of groups tends to be the same, in which the increase of the IgG level occurs after the therapy. However, if the increase of IgG level in both of groups was compared to each other, it would show that the increase of IgG level in the treatment group was bigger than in the control group. The increase of IgG level in the treatment group was about 96.1 mg/dL; meanwhile, the increase of IgG level in the control group was only about 85.6 mg/dL.

**DISCUSSION**

Until now there are still many problems in aggressive periodontitis cases related to its treatment and prognosis. The reason is because the causes of aggressive periodontitis are not only from the local factors. The condition of the patients related to the abnormal immunity of the body also has an important role. Therefore, immunoglobulin-G of the perifer of blood sample is used as a measurement of the immunity of the body in this study.

Based on the result data of aggressive periodontitis patients, the level of IgG was affected by clindamycin.

**Table 1.** Mean and standard deviation of IgG Level

IgG	Treatment $\bar{X} \pm SD$		Control $\bar{X} \pm SD$	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
	1392.3 ± 7.793	1488.4 ± 54.425	1420.8 ± 73.155	1506.4 ± 76.946

The fact that clindamycin affects the immunity status of the body was proven by the increase of IgG level. Nevertheless, the effect caused by clindamycin has the same pattern as tetracycline + metronidazole in the control group. Moreover, the data result slightly does not reflect the affectivity of clindamycin, but if it is analyzed further, it will show that the period of the therapy with clindamycin is shorter than that of the therapy with the other medicines in the control group. The therapy with clindamycin only needs 7 days, so the period of the treatment is shorter and the side effect of consuming the medicine can also be minimized. Meanwhile, the therapy with the other medicines in the control group needs 22 days.

IgG actually is not only a main component of immunoglobulin serum, but also a kind of immunoglobulin which has most amount in blood. This antibody also has a role in preventing formation and colonization of bacteria in tissues, in improving the function of phagocytosis through opsonisation process, and in helping detoxification process of toxin produced by bacteria. For those reasons, IgG has a potential role in body immunity.<sup>23</sup>

Furthermore, even though the increase of IgG level occurs in post therapy, the increase was still in normal level. Therefore, the increase was not considered as the abnormal one which can cause the negative effect for the body. The highest level of IgG after the therapy was 1574 mg/dL; meanwhile, the normal level of IgG was between 800–1700 mg/dL. Thus, the increase beyond the normal level must be prevented.

In addition, there are some possibilities of the increase of IgG level. First, the increase of IgG level may be caused by the chemical bound between antibiotic and carrier protein, so they become immunogenic. Immunogens can stimulate the immune responses. Immunogens in B cell can cause proliferation and differentiation of B cell into plasma cell together with immunoglobulin result. This mechanism occurs since B cell acts as antigen presenting cell (APC), so immunogens can directly be bound by B cell. Antibiotics can become immunogenic if there is the chemical bound between antibiotics and carrier protein. The immunogens recognized by B cell then pass proliferation and differentiation process, so it can stimulate the formation of antibodies.<sup>23</sup>

Another possibility is that the increase of IgG level after the therapy may be caused by the chemical bound between the medicines and macrophages. The immunogens which are from the kinds of medicines can be bound with macrophage which function is also as APC. As the explanation before, clindamycin is also able to distribute into mononuclear phagocyte cells. Thus, with the chemical bound between the medicines and macrophage it then can stimulate the immunity responses.

In addition, the increase of IgG level may also be caused by the effect of inflammation mediator, Prostaglandin-E<sub>2</sub> (PGE<sub>2</sub>). PGE<sub>2</sub> is arachidonate metabolic acid produced either by monocyte/macrophage cell or by fibroblasts.<sup>24</sup> The macrophage cell which is activated will produce IL-1, TNF and PGE<sub>2</sub>. In this study, moreover, the inflammation

causes the increase of PGE<sub>2</sub> level. The reason is because the condition of inflamed tissues can activate phagocyte cells which then cause the excretion of inflammatory mediator, PGE<sub>2</sub>. Moreover, according to Harris, Prostaglandin-E<sub>2</sub> has double actions. In the high level, PGE<sub>2</sub> can decrease the level of IgG; meanwhile, in the low level, PGE<sub>2</sub> can join IL-4 which then can increase the level of IgG deliberately. For those reasons, the low level of IgG in this study may be caused by the high level of PGE<sub>2</sub>. Thus, it not only can inhibit the activation and proliferation of B lymphocyte by T lymphocyte (CD4<sup>+</sup>), but can also inhibit the differentiation of B cell into plasma cell, so it also causes the inhibition in producing antibodies.<sup>9</sup>

After the clindamycin therapy, the increase of the IgG level occurred. The reason was because of the ability of antibiotics, clindamycin, in eliminating bacteria causing infections, so it then could reduce the level of the inflammation. The decreasing of the inflammation level then will be followed by the decreasing of the inflammatory mediator production, including PGE<sub>2</sub>. The low level of PGE<sub>2</sub>, therefore, can reduce the inhibition of proliferation and differentiation of B lymphocyte into plasma cell, so the production of antibodies (immunoglobulin) by plasma cell will increase.

The increase of the IgG level is needed since IgG has a role in opsonisation of antigen in phagocytes process, so the immunity mechanism can become more effective. The increase of the IgG level in this study, thus, indicates that clindamycin has a role in increasing humoral immunity. In other words, it can cause aggressive periodontitis patients who follow the therapy with clindamycin can not be susceptible to diseases since there is the increase of the body immunity.

Based on the above explanation, it may be concluded that clindamycin can increase the level of IgG since clindamycin has immunomodulatory character through the controlling mechanism of pro-inflammatory mediator. However, tetracycline with metronidazole can also role as immunomodulatory, but clindamycin more efficient than those because treatment with clindamycin needs single drug only so multiple drug side effects can be minimized. Finally, clindamycin can be used as drug of choice for the treatment of aggressive periodontitis since clindamycin can improve the immunity status of aggressive periodontitis patients.

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