Anti-Cardiolipin Antibodies in Chronic Periodontitis Patients in Kelantan, Malaysia

Wan Majdiah Wan Mohamad¹*, Noor Rashidah Saad², Haslina Taib¹, Siti Lailatul Akmar Zainuddin¹

1. School of Dental Sciences, Universiti Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia.
2. Klinik Pergigian Baling, Jalan Weng, 09100, Baling, Kedah, Malaysia.

Abstract

This study aimed to determine and compare the levels of anti-cardiolipin (anti-CL) antibodies between chronic periodontitis (CP) and control (non-CP) patients. A cross-sectional study was conducted on 35 CP (based on pocket depth >3mm) and 39 non-CP patients aged 18 to 65 years old. Plaque Index (PI) and Gingival Index (GI) were recorded. Five millilitres of blood samples were taken for determination of immunoglobulin G (IgG) and immunoglobulin M (IgM) anti-CL antibodies level by using Enzyme Linked Immunosorbent Assay (ELISA) method. Data were analysed by SPSS version 20.0 with the significant P value of <0.05 at 95% CI.

PI and GI were found to be significantly higher in CP compared to non-CP group (P<0.05). The mean IgG anti-CL antibodies were significantly higher (P=0.002) in CP patients [4.46 (SD1.89)] compared to non-CP group [3.22 (SD1.35)]. The mean IgM anti-CL antibodies were also significantly higher (P=0.019) in CP patients [3.28 (SD:1.57)] compared to non-CP group ([2.57 (SD:0.91)].

We hypothesized that periodontal infections stimulates production of anti-CL antibodies through the molecular mimicry mechanism.

Keywords: Anti-cardiolipin antibodies, chronic periodontitis, serum immunoglobulin.

Received date: 25 March 2017
Accept date: 16 May 2017

Introduction

Cardiolipin is a phospholipid (diphosphatidylglycerol) located in the inner mitochondrial of mammalian membranes which is found in mammalian tissue, eukaryotic organism and some prokaryotic bacteria.¹

It plays as an integral role as normal electron transport and energy metabolism.² Cardiolipin can start an antibody response in various diseases involving mitochondrial damage.³

Antibodies to cardiolipin are part of family of auto-antibodies directed against cell membrane phospholipids which are known as anti-CL antibodies. They are found in 1% to 5% of systemically healthy individuals⁴ and can increase in several infectious diseases.⁵ These antibodies are found commonly in individuals with Systemic Lupus Erythematos (SLE) or Anti-Phospholipid Syndrome (APLS).⁶ These prothrombotic auto-antibodies also are associated with adverse pregnancy outcomes such as fetal involution, prematurity, low birth weight with cardiovascular sequelae such as atherosclerosis, stroke and myocardial infarction.⁵ All of these conditions are remarkable similar to systemic condition associated with periodontitis.

Anti-CL antibodies can be classified in subclasses as IgM, IgG and IgA or as β2–glycoprotein dependent or independent. In β2–glycoprotein dependent group, anti-CL antibodies require presence of β2GPI to bind to cardiolipin such as in autoimmune diseases like SLE or APS. Meanwhile, in patient with syphilis or other infectious diseases the antibodies react directly; they are not only independent but also inhibited by β2GPI.⁷

β2GPI is a plasma protein that binds to negatively charged phospholipid and is thought to have protective homeostasis mechanism by

*Corresponding author:
Dr Wan Majdiah Wan Mohamad
MD (USM), M(Path) USM
Lecturer in Pathology (Clinical Immunology)
School of Dental Sciences
USM Health Campus
16150 Kubang Kerian
Kelantan
E-mail: wmajdiah@usm.my
preventing pathological prothrombotic reaction by platelets or endothelial cell.⁹

Chronic periodontitis (CP) is one of the most prevalent chronic diseases affecting people worldwide characterized by gingival swelling, loss of alveolar bone and movement of the teeth.⁹ World Health Organization (WHO) reported that 15% to 20% of the world population aged 35 to 44 years suffered from advanced periodontitis.¹⁰

It is the most common form of periodontitis which has increased prevalence in adults, slow to moderate in progression, presence of sub gingival calculus, its severity is consistent with local factors, and usually associated with a different type of microbial pattern. It is well known that a specific group of microorganisms leads to continuous destruction of the periodontal tissue results in a detectable clinical attachment loss, accompanied by pocket formation, gingival recession, as well as bone resorption.¹¹

The main aetiology of this chronic inflammatory condition is bacterial plaque modified by various risk factors such as smoking, diabetes, stress, drugs, systemic disease, and nutrition; while the risk determinants includes genetics, socioeconomic status and gender.¹²

Previous studies¹³,¹⁴ reported that presence of association between systemic conditions and periodontitis, most probably due to the effect of periodontal pathogens especially gram negative bacteria such as *Porphyromonas gingivalis*, which induced production of systemic inflammatory mediators. Some researchers proposed that increase in systemic markers of endothelial inflammation is associated with increase in serum levels of auto-antibodies including an anti-CL antibodies.⁵,¹⁴,¹⁵ It has been proposed that the association between periodontal disease and anti-CL antibodies are through viral and bacterial infections that induce production of these antibodies via molecular mimicry mechanism.¹⁶,¹⁷

Although various evidence from previous studies⁵,⁸,¹⁴,¹⁵,¹⁸-²⁰ have suggested the association between anti-CL antibodies and periodontal disease, the detectable anti-CL antibodies in chronic periodontitis is remain uncertain. Thus this study was conducted to determine the level of the IgG and IgM anti-CL antibodies among chronic periodontitis patients and then compared with those without periodontitis.

Materials and methods

This cross sectional study has been carried out on 35 CP patients from Periodontal Clinic, Hospital Universiti Sains Malaysia (Hospital USM). Thirty-nine of non chronic periodontitis (non-CP) patients were recruited as control. Diagnosis of chronic periodontitis was based on the periodontal classification of the American Academy of Periodontology.²¹

The selected age ranged was from 18 to 65 years old without any medical problems. Patients with aggressive periodontitis, pregnancy and lactating mothers, smokers, as well as those who suffered from any chronic medical conditions such as diabetes mellitus, hypertension, rheumatoid arthritis, respiratory infection, and bleeding disorders were excluded from the study. Plaque Index (PI)²² and Gingival Index (GI)²³ were recorded for all subjects. Five millilitres of venous blood was withdrawn from ante-cubital fossa of all subjects. The blood then was transferred into plain bottle and was sent to the laboratory immediately. The samples were allowed to clot for one hour at room temperature before centrifugation for 5 minutes at 4,500 rpm (Hettich Zentrifugen Universal 32 R Centrifuge) at 4°C. The sera were separated, placed in vials and stored at -80°C until assayed for IgG and IgM anti-CL antibodies. This study protocol was approved by local ethics review board; Human and Research Ethics Committee, USM (USMKK/PPP/JEPeM[232.3.(01)]). Informed written consents were obtained prior to the data collection procedures.

Laboratory procedures

The measurement of IgG and IgM anti-CL antibody levels was performed according to the manufacture’s protocol using the commercially available ELISA kit. Reported levels of anti-CL antibodies were expressed in GPL Unit/ml for IgG and MPL Unit/ml for IgM anti-CL antibodies. The interpretation of the anti-CL antibodies levels was based on the reference values as follow: Normal IgG < 10GPL Unit/ml and normal IgM <7MPL Unit/ml.

A sufficient number of microplate modules were prepared to accommodate controls and prediluted patients’ samples. A 100µl of calibrators’ controls and prediluted patients’ samples were pipetted in duplicate into the microwells. The microwells were incubated for 30 minutes at room temperature. Following
incubation, the contents of microwells were discarded and washing was carried out for three times with 200µl of wash solution. A 100µl of enzyme conjugate was dispensed into each well. Following incubation for 15 minutes at room temperature, the contents of the microwells were discarded and washing was carried out for three times with 200µl of wash solution. A 100µl of Thiomethylene Blue (TMB) substrate solution then was dispensed into each well. Incubation for another five minutes was to allow production of a yellow colour end product. The optical density was read at 450nm within 30 minutes.

Statistical Analysis
All data were analysed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software. P value of less than 0.05 was considered as statistically significant. Descriptive statistics of demographic variables were expressed as frequency and percentage while variables for the levels of IgG and IgM were expressed in mean (SD) Unit/ml. Independent t-test was used for analysis of mean comparison of IgG and IgM anti-CL antibodies for both CP and non-CP groups.

Results
General characteristics of the study subjects
A total of 74 subjects was enrolled in this study, with mean age of 43.23 (SD11.40) and 34.97 (SD10.88) years for CP and non-CP groups respectively. Majority of the subjects were Malays whereby female outnumbered male patients in both groups (Table 1). Table 2 shows the periodontal parameters (PI, GI, PPD, and CAL) in CP and non-CP groups. All parameters were significantly higher in CP compared to non-CP (p<0.05).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non Periodontitis (n=39)</th>
<th>Chronic Periodontitis (n=35)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>0.51 (0.34)</td>
<td>0.69 (0.55)</td>
<td>0.001</td>
</tr>
<tr>
<td>GI</td>
<td>0.47 (0.25)</td>
<td>0.59 (0.46)</td>
<td>0.000</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>1.71 (0.33)</td>
<td>2.66 (0.83)</td>
<td>0.000</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>0.14 (0.29)</td>
<td>1.16 (1.33)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2. The periodontal parameters of the study subjects (n=74).

Serum anti-CL antibody levels in chronic periodontitis and non-chronic periodontitis
Table 3 shows the level of IgG and IgM anti-CL antibodies in CP and non-CP groups. According to reference value, the levels for both IgG and IgM were within the normal range. However the mean levels of both anti-CL antibodies were significantly higher in CP compared to non-CP group. The mean level of IgG anti-CL antibodies were 4.46 (SD1.89) and 3.22 (SD1.55) in CP and non-CP groups respectively. Meanwhile, the mean level of IgM anti-CL antibodies in CP group was 3.28 (SD1.57) and non-CP group was 2.57 (SD0.91). Analysis using Independent t-test showed that the level of IgG and IgM anti-CL antibodies were significantly higher in CP compared to non-CP group with the $P$ value of 0.002 and 0.019 for IgG and IgM anti-CL antibodies respectively. Nevertheless, the mean levels of anti-CL antibodies for both groups did not exceed the reference normal value.

Table 3. Mean Comparison of anti-CL antibodies levels between NP and CP groups.

Discussion
In this present study, the anti-CL antibodies of 35 patients with CP were compared with 39 patients without the disease. The mean age of CP patients was much older than non-periodontitis subjects which reflects that periodontitis is commonly occur in older age
group. Majority of the selected patients in both groups were female. Gonsalves et al.\textsuperscript{24} reported that elderly patients have a risk of developing chronic diseases of mouth including dental caries and periodontitis. A report by National Oral Health Survey of Adult in Malaysia (NOHSA)\textsuperscript{25} found that as the age increased, the proportion of subjects with healthy periodontium is also decreased. The prevalence and severity of periodontal disease was found to be increased with age.\textsuperscript{26} In addition, Horning et al.\textsuperscript{27} reported that age is the risk factor for periodontitis although the main cause of attachment and bone loss is the presence of plaque and calculus. Even though the increase in age was related to increase in susceptibility of patients toward chronic periodontitis is still inconclusive, the changes of host immune response and the cumulative effects of disease over the lifetime might explained the periodontal disease prevalent in elderly population.\textsuperscript{28}

Malay is the largest ethnic group in Kelantan followed by Chinese. This could be the reason most of the subjects participated were Malays (94.9\%) and only 5.1\% were Chinese. Similar observation was described by Normastura et al.\textsuperscript{29} in their study in which they found that 98.1\% of their study subjects were Malays and only 1.9\% was Chinese.

The interrelationship between periodontal disease and systemic diseases becomes a common topic of interest by many researchers nowadays. The underlying mechanisms and shared risk factors in both conditions have been explored and explained in previous studies.\textsuperscript{18, 19} Despite vast studies in this area, the involvement of anti-CL antibodies in both diseases is one of the issues that still need further clarification.

In this present study, the level of both IgG and IgM anti-CL antibodies were found significantly higher in chronic periodontitis compared to non-periodontitis subjects. Faghihi et al.\textsuperscript{18} reported that serum anti-CL antibodies was significantly higher in periodontitis group compared to control (non-periodontitis) group. This is similar with our findings. However, they had classified their subjects into generalized and localized chronic periodontitis and the levels of anti-CL antibodies were expressed in total. Corresponding to our result, Gunupati et al.\textsuperscript{19} also demonstrated that serum IgG and IgM anti-CL antibody levels in chronic periodontitis with acute myocardial infarction were significantly higher compared to non periodontitis subjects with acute myocardial infarction.

Meanwhile, Kumar \textsuperscript{8} reported that severe periodontitis showed a marked increase in IgG and IgM anti-CL antibodies compared to non-periodontitis, mild periodontitis and moderate periodontitis groups. The severe periodontitis patients showed a significant increase ($p<0.0001$) in both IgG and IgM anti-CL antibodies compared to other groups, thus suggested that those patients were predisposed to have systemic problem mainly coronary heart disease. A more recent study by Al-Ghurabei\textsuperscript{15} revealed that serum level of IgG anti-CL antibody was found significantly higher in chronic periodontitis than healthy group whereas there was no significance differences was found between the two groups for IgM anti-CL antibody level.

The possible explanation for the higher level of serum anti-CL antibodies among chronic periodontitis patients in our study could be due to the fact that infectious disease including periodontitis may induce the production of these antibodies. Salari and Kadkhoda\textsuperscript{30} reported several common pathogens that can cause periodontitis include \textit{Aggregatibacter actinomycetemcomitans} (26.8\%), \textit{Porphyromonas gingivalis} (21.9\%), \textit{Capnocytophaga sp} (16.7\%), \textit{Eikenella corrodens} (13.2\%) and \textit{Prevotella intermedia} (10.5\%). In addition, Wanget al.\textsuperscript{31} found that anti-CL antibodies were significantly higher in patients with positive \textit{Aggregatibacter actinomycetemcomitans}.

Recent studies strongly correlate bacterial and viral infection in the etiology of APS due to induction of cross-reactivity anti-CL autoantibodies. Blank et al.\textsuperscript{16} and Schenkein et al.\textsuperscript{14} have proven that anti-CL antibodies can be stimulated by bacterial pathogens such as \textit{Porphyromonas gingivalis}, \textit{Hemophilus influenza} or \textit{Neisseria gonorrhoeae}, and cytomegalovirus, which have peptide sequences similar to the TLRVYK peptide of $\beta2$GPI\textsuperscript{90}. Three peptides sequence that are present in the arg-gingipain protease of the periodontal pathogen \textit{Porphyromonas gingivalis} is similar to the TLRVYK peptie of $\beta2$GPI and can induce cross-reactive autoantibodies patients with periodontitis.\textsuperscript{14, 33}

In addition, periodontal bacteria including \textit{Aggregatibacter actinomycetemcomitans} and...
Porphyromonas gingivalis have been found to contain a peptide sequence similar to that on the β2GPI molecule, which is immunogenic and can provoke the production of anti-CL antibodies and may lead to chronic inflammatory reaction. Based on reference normal value of the anti-CL antibodies, this present study indicates both IgG and IgM anti-CL antibody levels were still within the normal range. This finding might be influenced by demographic and geographic factors where the prevalence and incidence of high anti-CL antibody levels in Malaysia is quite low. Previous study by Jones et al. reported a low prevalence of positive anti-CL antibodies (16.5%) in 200 SLE patients attending Kuala Lumpur University Hospital, Malaysia. By contrast, few studies reported among European patients with SLE, the prevalence of anti-CL antibodies varies from 39.0% to over 60.0%. Meanwhile, another study in Malaysia reported the incidence of positive IgG anti-CL antibodies in SLE patients was 13.7%, but higher incidence of such antibodies was found in primary and secondary APS which is 36.4% and 40% respectively.

In 2003, Schenkein et al. reported the prevalence of anti-CL antibodies in chronic periodontitis and generalized aggressive periodontitis patients were 16.2% and 19.3% respectively. The lower prevalence was observed in healthy (6.8%) and localized aggressive periodontitis (3.2%). They also proposed that greater extent and severity of the disease as well as inflammation may lead to production of anti-CL antibodies in those patients. The lack of anti-CL antibodies in localised aggressive periodontitis could be due to the difference in periodontal pathogen involved in localised and generalised disease. In addition, they also found that elevation of anti-CL antibodies particularly in generalized aggressive periodontitis is associated with elevated levels of systemic markers of vascular endothelial inflammation.

Conclusions

Elevated anti-CL antibody levels in chronic periodontitis patients suggests that oral or periodontal infections may involve in the stimulation of the anti-CL antibodies production. This may lead to the development of infective endocarditis, coronary heart disease, thrombosis and stroke. Therefore anti-CL antibodies may serve as an important marker in relation to dental and systemic disease. Thus, early intervention or management could be taken with early detection of anti-CL antibodies in the serum to prevent further complication of the disease.

Acknowledgements

We would like to express our gratitude to all patients and staff who involve direct or indirectly in this study. This study was supported by Universiti Sains Malaysia Short Term Grant (304/PPSG/61311040).

Declaration of Interest

The authors report no conflict of interest.

References


