An assessment of anti-citrullinated protein antibody in systemically healthy individuals with or without chronic periodontitis: A case–control study

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INTRODUCTION

Chronic periodontitis (CP), inflammation of the supporting tissues of teeth, often is accompanied by progressive destruction of the periodontium, followed by severe sequelae such as pocket formation, recession, and eventually tooth exfoliation.[1,3] Periodontal disease (PD) not only causes the loss of tooth but also has been shown to influence the systemic health of the individual[3] and may also contribute to disorders such as diabetes, respiratory, cardiovascular, cerebrovascular diseases, osteoporosis and metabolic syndrome as well as rheumatoid arthritis (RA).[2,4] RA leads to systemic inflammation at a chronic level, affecting adversely the cartilage and bones pertaining to small joints of the limbs. This may cause contrasting degrees of deformity and functional disability.[5] The ability of RA to inflict similar damage to the peripheral joints reduces the quality of life to a great extent.[4] PD was found affiliating with RA, despite the fact that the etiologies of the two conditions are different.[6] The reason may be attributed to the similarity of the underlying pathological processes, such that individuals in danger of acquiring one condition are also at risk for the other.[6]

Abstract:

Background: Periodontitis has been implicated as a risk factor for rheumatoid arthritis (RA). Aim: This study aimed to assess the relationship between RA and chronic periodontitis (CP) by evaluating the serum levels of the anti-citrullinated protein antibody (ACPA) which is a marker of RA in systemically healthy individuals with and without CP. Materials and Methods: This case–control study enrolled 40 systemically healthy individuals. Participants were divided into two groups, i.e., CP group Systemically healthy chronic periodontitis (CPSH) (n = 20) and control group Systemically healthy (SH) (n = 20), matched for age and gender. The CP patients were evaluated for periodontal parameters, namely probing pocket depth, clinical attachment loss, percentage of the site involved with attachment loss, and number of teeth present. A volume of 5 ml of venous blood was collected from both the groups and centrifuged; the separated serum was stored at − 70°C before being analyzed. Later, serum samples were tested for levels of ACPA in both the groups and compared. Results: The mean serum ACPA levels were higher in CPSH patients compared to SH (131.38 RU/ml vs. 34.54 RU/ml, P = 0.001), which was statistically highly significant. In addition, we found a significant elevation of serum ACPA levels in severe generalized CP patients compared to moderate generalized CP patients (175.47 RU/ml vs. 95.31 RU/ml, P = 0.001), and the difference was statistically highly significant. Conclusion: The results of the study confirmed that CP can be a risk factor for RA. Moreover, the severity of periodontitis appeared to be related to elevated serum levels of ACPA.

Key words:

Anti-citrullinated protein antibody, chronic periodontitis, rheumatoid arthritis

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RA is an autoimmune disease, wherein substances such as fibrinogen, vimentin, collagen Type II, and alpha-enolase, identified as autoantigens, are found to be present in the joints.[7] In reaction to these autoantigens, autoantibodies such as anti-citrullinated peptide antibody (ACPAs) develop, which was previously called anti cyclic citrullinated protein (anti-CCP), leading to subsequent autoimmune damage.[7,9]

Porphyromonas gingivalis (Pg) was found significantly present in periodontitis, and it also correlates with the occurrence of RA.[8] Pg exclusively expresses citrullination enzyme peptidylarginine deiminase (PPAD) that causes the posttranslational modification and catalyzes the citrullination of protein arginine to citrulline.[3,9]

Antibodies associated with RA have also been detected in periodontitis patients not suffering from RA.[9] Inflamed periodontal tissues also show increased levels of cyclic citrullinated proteins (CCPs), along with the presence of ACPAs in gingival crevicular fluid (GCF), saliva, and serum of patients of RA.[10-12]. The diagnosis of RA routinely involves the detection of ACPA, using enzyme-linked immunosorbent assay (ELISA) based on CCPs.[13] Several studies have shown that it is sensitive and is a 95%–98% specific diagnostic marker of RA.[9]

Hence, in this study, the levels of ACPA in systemically healthy individuals with or without CP were assessed.

MATERIALS AND METHODS

Source of data
A case–control study was performed. Individuals were selected from among patients reporting to the outpatient department of periodontology. The study involved a total of forty participants that included twenty cases of periodontitis (severity – moderate to severe [CPSH]) and twenty healthy individuals (SH). Matching for both age and gender was carried out.

Informed written consent from the study participants was obtained. Ethical clearance was obtained from the institutional ethical committee.

Power analysis suggested that forty patients with the power of 80% would provide sufficient data for appropriate statistical analysis, along with 0.81 as the effect size, so as to detect significant differences.

Inclusion criteria
A. Test group: Patients diagnosed with moderate-to-severe generalized CPSH (American Academy of Periodontology 1999).[14] >30% of the sites involved with ≥3 mm of clinical attachment loss (CAL), radiographic evidence of bone loss with orthopantomogram (OPG), and individuals having maximum twenty numbers of teeth present excluding the third molar in CP group.
B. Control group: Individuals with ≤3 mm probing pocket depth (PPD) and no CAL.

Exclusion criteria
Patients with a history of diabetes mellitus, asthmatics, hypertension, or RA or any other systemic diseases, pregnant patients or lactating mothers, patients with a history of alcohol consumption, patients with a history of periodontal treatment in the last 6 months, patients undergoing any antibiotics or anti-inflammatory therapy in the past 3 months or on hormonal therapy, patients with a history of radiation therapy, smokers, and patients with localized or generalized aggressive periodontitis.

Clinical parameters
These were measured by the University of North Carolina-15 periodontal probe. The PPD and CAL from six sites on each tooth were recorded to determine the periodontal status. OPG was taken to assess the extent of bone loss.

Blood sample collection
The blood sample was collected from the antecubital vein, i.e., 5 ml in a sterile test tube from both the groups. Centrifugation of the blood was carried out after coagulation, and the serum was stored at −70°C until processed.

ACPAs level estimation
Commercially available ELISA kit (Biogenix®) [Figure 1] was used to assay for ACPA of the serum samples, used as per the instructions of the manufacturer, and readings were recorded through ELISA reader [Figure 2] which gave optical density values; later, values were converted in relative units per milliliter (RU/ml).

Statistical analysis
SPSS version 20 (IBM SPSS statistics [IBM Corp., Armonk, NY, USA released 2011]) was employed for data analysis. Mean and standard deviation were computed to express the quantitative data, and Student’s paired t-test was employed to distinguish two quantities between the two groups. P < 0.05 was considered as statistically significant, whereas P < 0.001 was treated as highly significant. The examiner standardization was done using the kappa coefficient (κ), and the variables were measured by the same examiner for the same thing which was taken at different time intervals.

The most likely values extend from +1 (perfect agreement) via 0 (no agreement above that expected by chance) to −1 (complete disagreement).

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κ = \frac{Po - Pe}{1 - Pe}
\]

Where Po − observed agreement and Pe − expected agreement.

RESULTS

From the total forty patients included in the present study, twenty were CPSH, whereas twenty were SH patients. Clinical periodontal examination included recording the PPD, CAL, percentage of sites with CAL, and numbers of teeth present [Table 1] and was recorded by the same examiner.

The mean ACPA was significantly higher in the CPSH group (131.38 ± 51.17) compared to the SH group (34.54 ± 24.45) (P = 0.00) [Table 2 and Graph 1].
The difference in mean scores of ACPA levels was significantly higher in the severe generalized CP group (175.47 ± 44.50) as compared to the moderate generalized CP group (95.31 ± 14.54) \((P = 0.001)\) [Table 3 and Graph 2].

**DISCUSSION**

RA and PD have a huge impact on public health and are found to affect a large population throughout the world.[15]

ACPAs specific to RA appear before the onset of disease, from the preclinical to the diseased stage, hence proving that ACPA levels may be used to predict the onset as well as the stage of severity of the disease.[16-18]

Various studies have been done where ACPA is found in smokers and systemic conditions such as asthma. Smoking is an established environmental factor which is associated with RA and periodontitis development and ACPA positivity in RA. It has been suggested that this may be due to increased expression of peptidylarginine deiminase (PAD) enzymes in the lungs of smokers leading to the citrullination of native proteins in the lung, which in turn results in the formation of ACPA.[19]

Even asthma may be a risk factor for RA-related autoantibody development before clinical presentation of RA is seen. Lung and respiratory tract have the most evidence in support of being an imitating site for RA. The biologic effect of smoking or other inhalants on RA risk may be due to induction of local pulmonary mucosal inflammation at the bronchioles and alveoli, leading to protein citrullination, loss of immune tolerance, and the apparent formation of ACPA preceding RA symptoms.[20]

Hence, in order to avoid bias, we excluded smokers and asthmatics from our study.
There is a need to conduct studies that correlate RA related antibodies and PD, as it has been shown that ACPA is found in not only diseased but also healthy population. Such studies can reveal the causality of PD on antibody production.\cite{14} Thus, the present study was performed to assess ACPA levels in the serum of healthy participants with and without CP.

In our study, when ACPA levels were compared, a statistically significant difference was established between the CPSH and SH groups (P < 0.01). In a similar study done by Lappin et al., significantly elevated levels of ACPA were expressed in the CPSH group compared to healthy participants. However, the ACPA level in CPSH was within the normal range of their diagnostic kit.\cite{12}

In the present study, we found an increased level of ACPA in the CPSH group, above the normal range of our diagnostic kit. These increased levels of ACPA are significant as it can be seen nearly 10–14 years before the onset of RA.\cite{16,12} These elevated ACPA levels indicate periodontitis as an important risk factor for RA development since it is now destructive to a greater extent.\cite{21}

Variations found in the ACPA levels between both the studies could be due to the utilization of different diagnostic kits or due to the difference between the numbers of teeth present.

Yang et al. stated that the extraction of teeth led to reduced levels of ACPA due to reduced bacterial load. ACPA is 95% specific for established RA cases with 40%–55% specificity in early-onset RA cases. Hence, we may assume that around 40%–55% of our cases are in early-onset RA.\cite{22}

Terao et al. observed that periodontal parameters such as community periodontal index and loss of attachment were associated significantly with an increased ACPA level in smokers and nonsmokers. The association of PD with ACPA production is supported by relevant correlations between PD parameters, positivity, and also levels of ACPA in a healthy population.\cite{14}

Lappin et al.\cite{12} and Yang et al.\cite{22} evaluated the effect of nonsurgical periodontal treatment (NSPT) of periodontitis on serum levels of ACPA in patients with CP. NSPT was found to significantly decreased serum ACPA in CP patients, especially in patients with generalized CP.

Nesse et al.\cite{23} observed the presence of citrullinated protein in 80% of periodontitis-affected stroma compared with 33% of healthy periodontal stroma.

Harvey et al.\cite{15} observed the citrullinated proteins, PAD-2 and PAD-4 as well as ACPA in inflamed periodontal tissues. They stated that as none of the participants in their study had RA, so the presence of ACPA in the GCF of patients may indicate preclinical phase of RA.

In our study, we even found a significant elevation of serum ACPA levels in severe CP patients compared to moderate CP. To our knowledge, no studies have been conducted to assess ACPA levels according to the severity of periodontitis, however, there is evidence to support the severity of PD that is related to the severity of RA.

Mercado et al. observed that in RA patients, moderate-to-severe CP was significantly prevalent. Moreover, in contrast to the general population, RA patients were regularly referred for periodontal treatment.\cite{30} A similarly increased prevalence of periodontitis was found by de Smit et al. in RA patients with the highest disease activity in patients with severe periodontitis. They also discovered certain serological markers for systemic inflammation common to the two diseases, hence reinforcing the connection between the two diseases.\cite{31}

In the present study, the probable explanation for higher serum levels of ACPA in CP, particularly with severe generalized CP, compared with moderate generalized CP and with the healthy controls, maybe because Pg is the pathogen that plays a crucial part in the pathogenesis of PD.

Takeuchi et al. showed that Pg can be detected in 80%–90% of periodontitis patients and 10%–30% of healthy participants.\cite{24} A link was proposed between PD and RA is interlinked through the role of Porphyromonas gingivalis because of its distinctive enzyme peptidylarginine deiminase (PAD).\cite{25-28} In their study, Lappin et al. compared CP patients with healthy controls. They presented increased anti-Pg antibody titers in the former group as compared to the latter.\cite{12} Milkus et al. identified levels of anti-Pg antibody, as being lowest in controls and intermediate in RA patients.\cite{29} Rosenstein et al.\cite{30} and Hitchon et al.\cite{31} found that a higher ACPA serum concentration yield elevated the anti-Pg antibody titers.

**CONCLUSION**

There was an increase in the ACPA levels in CP patients. Furthermore, the severity of periodontitis was found to be related to the elevated serum levels of ACPA. Thus, it can be said that individuals suffering from CP could experience RA in the future. The levels of Pg were not measured in the present study; therefore, investigations for the presence of this microorganism should be conducted to obtain more accurate results. Hence, a further long-term prospective and interventional study with a larger sample size is needed.

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**Table 3: Comparison of anti-citrullinated protein antibody in moderate and severe generalized chronic periodontitis groups**

|             | n     | Minimum | Maximum | Mean | SD   | Mean difference | P     |
|-------------|-------|---------|---------|------|------|-----------------|-------|
| Moderate CPSH | 11    | 59.60   | 112.00  | 95.31| 14.54| −80.15          | 0.001*|
| Severe CPSH  | 9     | 140.25  | 290.00  | 175.47| 44.50|                 |       |

CPSH – Chronic periodontitis group; SD – Standard deviation; n – Total number of patients; P – Probability. P<0.05 was considered as statistically significant. *P<0.001 was considered as highly significant.
Conflicts of interest
There are no conflicts of interest.

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