RESEARCH ARTICLE

Case-control study for assessment of factors associated with periodontitis among adults attending a university hospital in Karachi, Pakistan

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Abstract

Objective: To compare the physiological and biochemical markers in healthy and periodontitis subjects, and to relate these markers with the periodontal health condition.

Methods: The case-control study was conducted at the Dow University of Health Sciences, Karachi, from April 2017 to March 2018, and comprised systematically healthy controls and periodontitis cases. Periodontal probing depth, clinical attachment loss, oral hygiene indices, educational status and body mass index were recorded for all the subjects. Serum levels of biochemical markers, including calcium, phosphate and interleukin-6, were also measured. Data was analysed using SPSS 16.

Results: Of the 150 subjects, 75(50%) each were in the case and control groups. The overall mean age was 31.23±3.7 years (range: 22-42 years). The cases had relatively poor oral hygiene indices and educational status compared to the controls ($p<0.05$). Serum calcium level was lower, whereas mean body mass index was higher in the cases compared to the controls ($p<0.05$). No significant difference was found in interleukin-6 and phosphate levels ($p>0.05$). Clinical attachment loss showed significant correlation ($p<0.05$).

Conclusion: Low serum calcium and educational levels, higher body mass index and poor oral hygiene were found to be the risk factors for the progression of periodontitis.

Keywords: Periodontitis, Serum calcium, BMI, Oral hygiene, Clinical attachment loss. (JPMA 71: 252; 2021)

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Introduction

Periodontitis is a chronic infection which occurs due to bacterial infection followed by host inflammatory response, leading to the demolition of the supporting tissues of the teeth.[1] The disease results in the detachment of gingiva, destruction of alveolar bone and is characterised by tooth mobility, gingival bleeding and formation of up to 4mm deep periodontal pockets.[1,2] This ailment affects about 20-50% of the total world population, but a comparatively higher occurrence has been seen in Asian countries, including Pakistan.[3,4] Several local studies have reported the prevalence of periodontal disease to be about 98%, with 31% having advanced periodontitis.[4,5]

Though all individuals are equally susceptible to periodontitis, certain risk factors increase the disease incidence, its severity and progression in prone individuals. An understanding of such factors is necessary for proper disease management and improved patient outcomes.[1,6,7] "Modifiable" and "non-modifiable" risk factors have been found to be associated with periodontitis and are widely discussed in literature.[1,6,8] Modifiable factors are usually controllable that can either be environmental or behavioural. Socioeconomic status (SES), psychological stress, smoking, alcohol consumption, nutritional status, microbial infections due to poor oral hygiene, diabetes mellitus (DM), cardiovascular disease (CVD), drug-induced disorders and obesity are some of the established modifiable risk factors for periodontitis.[1,8]

Non-modifiable factors are inherent aspects of an individual that can hardly be changed. These include genetic factors, host immune response, haematological disorders, hormonal changes, antioxidant imbalance, pregnancy, osteoporosis and ageing.[1,8]

Micronutrients, such as vitamins and minerals, are known to play an important role in maintaining good bone health and keeping strong immunity. As the diagnosis of periodontitis depends only upon disease history and clinical examination, serum levels of micronutrients could aid in the prediction of the prognosis of periodontitis. Pakistan National Nutrition Survey, 2011 revealed severe micronutrient insufficiencies among children, pregnant and nursing women.[9]

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The current study was planned to identify various modifiable risk factors that predispose the adult population in an urban setting for developing periodontitis.

Subjects and Methods

The case-control study was conducted at the Dr. Ishrat-ul-Ebad Khan Institute of Oral Health Sciences (DIKIOHS), Dow University of Health Sciences (DUHS), Karachi, from April 2017 to March 2018. After approval from the institutional review board, the sample size was calculated using Open-Epi version 3[10] in the light of an earlier study[4] conducted in Pakistan. Confidence interval was set at 95%, hypothetical proportion of controls with exposure 65% and power 80%. Additional subjects were included to avoid attrition. The sample was raised using non-probability purposive sampling technique. Those included were subjects aged 22-42 years. Those excluded were individuals with systemic conditions, such as DM, ischaemic heart diseases, hepatic diseases, renal diseases, those having received periodontal therapy within the preceding year, those who were using systemic antibiotics currently or within the preceding three months, subjects receiving vitamin D and calcium supplements, patients on radiotherapy or chemotherapy, periodontitis involving wisdom teeth, pregnant, lactating women and smokers. The case group comprised periodontitis patients who exhibited ≥2mm of clinical attachment loss (CAL) at one or more sites. Healthy subjects having ≤3mm periodontal probing depth (PPD), no bleeding on probing, no CAL and no evident bone loss on bitewing radiographs were taken as controls.

After informed consent, demographic data, including age, gender and level of education, was recorded for each subject. The study protocol was in line with literature.[11] A full mouth clinical examination was done using Michigan's probe.

For gingival, plaque, calculus and mobility indices, the index teeth of all subjects were recorded. Gingival and plaque measurements were done using Loe and Silness Index,[11] whereas calculus and mobility were determined using Green and Vermilion Index and Miller Index[11] respectively. The amount of CAL was used to classify the severity of periodontitis as mild (CAL= 1-2 mm), moderate (CAL= 3-4 mm) and severe (CAL = ≥5 mm)[11].

The height of the participants was measured in inches while weight was noted in kilograms using a mechanical scale. The body mass index (BMI) was calculated as the ratio of the individual's body-weight to the square of their height in meters. Based on World Health Organisation (WHO) guidelines for Asian populations, the subjects were categorised as underweight (BMI= <18.5 kg/m^2), normal (BMI= 18.5-22.9 kg/m^2) and overweight/obese (BMI= ≥23 kg/m^2).[12,13]

For laboratory measurements, 5ml venous blood sample was collected from each subject by standard venipuncture and the serum was stored at -70°C till analysis. Levels of serum phosphate were analysed using Quimica Clinica Aplicada (QCA) kit (Quimica Clinica Aplicada, Spain). Serum calcium levels were measured by colorimetric method using Dialab Diagnostics Kit (Austria). Interleukin-6 (IL-6) levels were measure using Human IL-6 enzyme-linked immunosorbent assay (ELISA_ Kit (Glory Science Co, USA).

Reference serum values for calcium was 8.5-10.8 mg/dl, and for phosphate 2.5-5 mg/dl.

Data was analysed using SPSS 16. Descriptive statistics was used for analysis and results were presented as mean ± standard deviation (SD). Frequencies and percentages were generated for categorical variables. Based on distribution of data, independent student's t-test and Mann-Whitney U test were used to compare the difference in variable means between the two groups. Chi-square test and Fisher's Exact test was used to compare categorical data. Biochemical parameters and BMI status according to the disease severity were analysed using analysis of variance (ANOVA) followed by post-hoc comparison. The relationship among serum calcium levels, BMI and CAL measurements was evaluated using Spearman's rank correlation. P<0.05 was considered statistically significant.

Results

Of the 150 subjects, 75(50%) each were in the case and control groups. The overall mean age was 31.23±3.7 years (range: 22-42 years). At baseline, difference in educational

Table-1: Baseline characteristics of cases and control, n=150.

Variables	Total (n=150)	Cases (n=75)	Control (n=75)	p-value
Age (years)[a]	31.2±3.7	31.6±4.4	30.8±2.8	0.21
Gender[b]				
Male	66.0 (44.0)	37.0 (49.3)	29.0 (38.7)	0.18
Female	84.0 (56.0)	38.0 (50.7)	46.0 (61.3)	
Education[b]				
High School Education	17 (11.3)	17.0 (22.6)	0.0 (0.0)	<0.001*
Higher Education	133 (88.6)	58.0 (77.3)	75.0 (100.0)	
Oral Hygiene[b]				
Good	118.0 (78.7)	44.0 (58.7)	74.0 (98.7)	<0.001*
Fair or Poor	32.0 (21.3)	31.0 (41.3)	1.0 (1.3)	
Periodontal Probing Depth (PPD), mm	3.0 (1.0-4.0)	4.0 (4.0-4.0)	1.0 (0.0-2.0)	<0.001*

Data given as mean+standard deviation (SD), number and percentage of subjects (n%) or median (lower quartile-upper quartile).
Inter-group comparisons were performed using: [a]Independent student's t-test, [b]Fisher's Exact Test.
*p<0.05=significant.

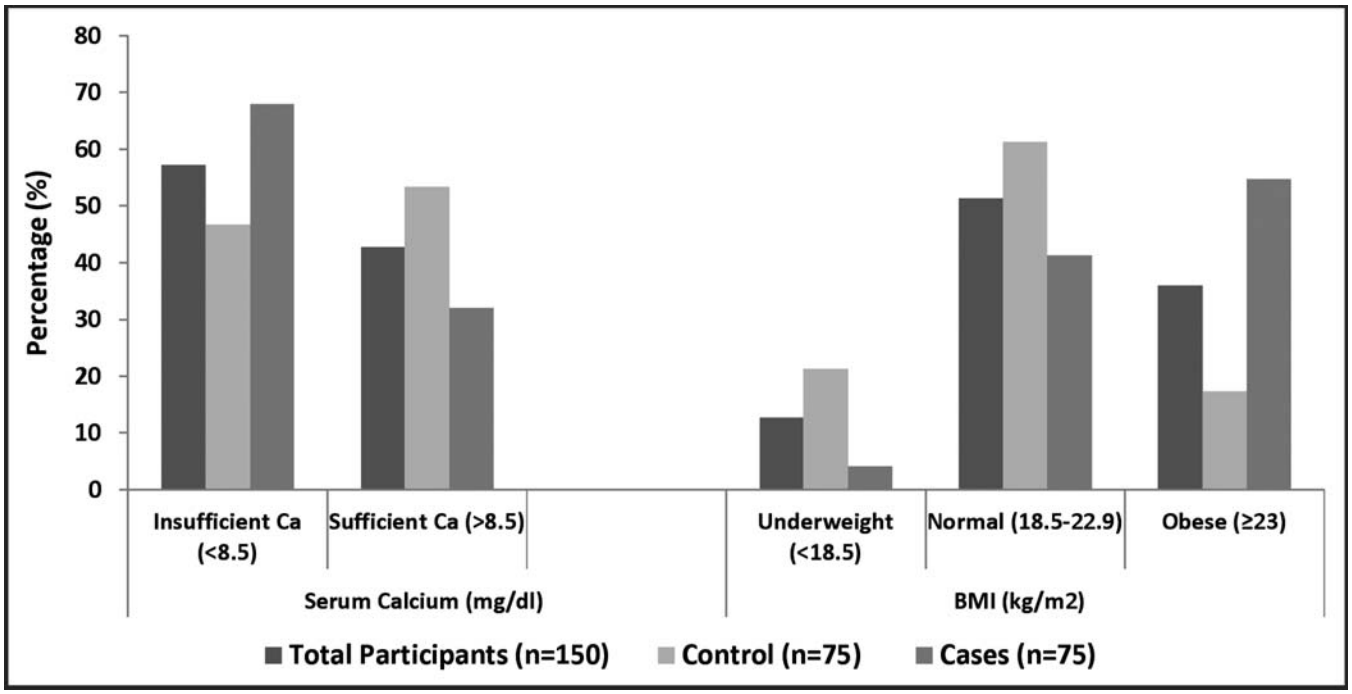

Figure: Serum calcium and body mass index (BMI) status.

Table-2: Physiological & biochemical health parameters of cases and control, n=150.

Variables	Cases (n=75)	Control (n=75)	p-value
Body mass index (BMI) (kg/m^2)[a]	23.5±4.0	20.7±3.4	<0.001*
Serum Calcium (mg/dl)[a]	8.2±1.8	8.8±1.0	<0.001*
Serum Phosphate (mg/dl)[a]	4.2±0.18	4.1±0.24	0.18
Serum interleukin-6 (IL-6) (ng/L)[c]	14.0 (1.0-71.0)	12.8 (2.0-29.2)	0.52

Data are given as mean±SD, or median (lower quartile-upper quartile).

[a] Independent student's t-test.

[c] Mann-Whitney U-test.

*p<0.05=significant.

Table-3: Body mass index (BMI) status and biochemical parameters in different periodontal health conditions, n=150.

Periodontal Condition based on CAL (mm)	n (%)	BMI (kg/m^2)	Calcium Levels (mg/dl)	Phosphate Levels (mg/dl)
Healthy (CAL=0)	75.0 (50.0)	20.7±3.4$^{\wedge}$	8.8±1.0	4.1±0.2
Mild (CAL=1-2)	9.0 (6.0)	26.0±5.8$^{\$}$	8.3±0.4	4.3±0.1
Moderate (CAL=3-4)	10.0 (6.7)	22.2±3.2	8.3±2.1	4.1±0.4
Severe (CAL≥5)	56.0 (37.3)	23.3±3.7$^{\$}$	8.1±1.9	4.2±0.1
p-value		<0.001*	0.09	0.15

Values are presented as mean±SD.

*p<0.05=significant.

$^{\wedge,\$}$ means different superscripts differ significantly.

CAL: Clinical attachment loss.

status, oral hygiene and PPD was significant between the groups (Table-1)

Calcium levels in periodontitis patients were significantly lower compared to controls (p<0.001). No significant difference was found in IL-6 and phosphate levels between the groups (p>0.05). BMI was significantly higher in the case than the controls (Table-2).

Calcium and BMI status of the participants was noted (Figure). No significant difference was observed in mean serum calcium levels in different periodontal conditions (p=0.09). Significant negative correlation between calcium levels with severity of periodontitis were found (p<0.05). Mean BMI was significantly different in different periodontal conditions (p=0.001) (Table-3). Additionally, significant positive correlation was observed between BMI and severity of periodontitis (p<0.05).

Discussion

Periodontitis is a bacterial infection-induced inflammatory disorder affecting the supporting tissues of the teeth, including connective tissue, alveolar bone and periodontal ligament, that can eventually lead to tooth-loss.[1] There are multiple other factors, including biochemical factors, that play an important role in disease progression.[6] Biochemical factors, such as serum inorganic calcium, phosphate and active vitamin D

metabolite (1, 25 vitamin D3) are thought to contribute in the development and progression of the disease either by affecting the periodontal structure or by interacting with the regulatory factors involved in the inflammatory response.[8,14] The current study evaluated various biochemical, inflammatory and oral hygiene related factors in healthy and periodontitis subjects to determine their role in disease development and progression.

Calcium is an important mineral, which is essential not only for the calcification of bones and teeth, but also plays a vital role in several physiological processes. Therefore, it is important for calcium levels to be tightly regulated, and in case of inadequate calcium intake, the body stimulates bone resorption to maintain the normal serum calcium levels.[15] Thus, calcium sufficiency is required to attain peak bone mass during growth and developmental stages and also to control the demineralisation of both skeletal and alveolar bones.[14,15] Calcium deficiency can affect the periodontium and weaken the tooth attachment, which can consequently lead to clinical manifestations of periodontitis.[15,16]

The current study found serum calcium levels in periodontitis patients to be significantly lower than those in healthy subjects. The low serum calcium levels observed in periodontitis subjects may be associated with insufficient intake of calcium, but the study did not record the calcium intake. The association between serum calcium levels and periodontitis in this study supported the findings that low calcium levels are one of the risk factors for periodontitis.[15,16] Studies have also reported a significant association of calcium intake and serum calcium in the progression of periodontal disease.[14,17,18] Contrary to our findings, some studies have reported increased serum calcium levels in periodontitis patients compared to controls.[19,20] The increased calcium levels observed in these studies was reflective of the negative calcium balance which results in calcium removal from the bones to restore the normal physiological levels. Chronic low intake of calcium may result in this negative balance and the resultant secondary increase in serum calcium levels.[16,21] Further studies are therefore required to assess the role of dietary calcium intake, serum and salivary calcium levels, and the metabolites involved in calcium homeostasis to understand the mechanism by which this cation contributes to the aetiology of periodontitis.

In the current study, subjects with periodontitis had relatively poor educational and oral hygiene status. The results were similar to the findings of studies showing that the poor oral hygiene status and low education increased the risk of periodontitis more than two-fold.[22,23] Additionally, it has also been reported that subjects who received oral hygiene instructions along with symptomatic treatment were less likely to suffer from chronic periodontitis.[19] Therefore, it is suggested that oral hygiene awareness should be given to the patients to reduce the risk of this prevalent disorder.

Obesity is a complex disorder characterised by an excess body-weight for height. It is a major health-related threat for the whole world that greatly contributes to the development and progression of numerous chronic disorders, including CVDs, DM, various cancers, and periodontitis.[24] According to the global disease burden research, Pakistan is among the top 10 obese countries of the world that is estimated to affect every 4th citizen.[25] BMI is the most commonly used matrix to assess obesity and categorising individuals into underweight, normal, and overweight/obese groups.

In the current study, subjects with higher BMI had a significantly higher likelihood to have periodontitis. On the other hand, majority of the periodontally healthy individuals were in the normal weight cluster. The results agreed with previous findings.[8,26,27] A few studies have also reported an inverse relationship between periodontal outcome and BMI which explains the involvement of multiple factors for both the diseases.[28,29]

Several explanations to link periodontitis and BMI have been provided.[27,30] Unhealthy dietary pattern rich in sugar and fat has always been ascribed for obesity. Impaired food habits contribute to poor oral health that exaccerbates the severity of periodontitis.[30]

Another possible mechanism that relates obesity and periodontitis is altered inflammatory state in obese persons. This immune dysfunctioning is reported to cause an imbalance in the cytokine network, especially an increased secretion of pro-inflammatory cytokines, such as IL-6, by adipose tissues which likely induce a hyper-inflammatory response on periodontitis.[31] Adaptation of a healthy diet with high nutritional value can control obesity, a modifiable risk factor, and help prevent its complications, including periodontitis.

Conclusion

Low serum calcium levels, low educational level, higher BMI and poor oral hygiene was found to be the risk factors for the progression of periodontitis. Serum calcium, oral health indices and BMI may be useful in evaluating periodontal health.

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