

# Effect of Non-surgical Periodontal Therapy on Serum Levels of TNF- $\alpha$ , IL-6 and C-reactive Protein in Periodontitis Subjects with Stable Coronary Heart Disease

Shuang Ying ZHOU<sup>1</sup>, Xiang Qing DUAN<sup>1,2</sup>, Rong HU<sup>3</sup>, Xiang Ying OUYANG<sup>1</sup>

**Objective:** To evaluate the effects of periodontal non-surgical therapy on serum levels of the inflammatory cytokines in chronic periodontitis subjects with stable coronary heart disease.

**Methods:** Seventy-five subjects with both chronic periodontitis (CP) and stable coronary heart disease (CHD) were enrolled in the study. Forty subjects received periodontal non-surgical treatment including oral hygiene instruction, scaling and root planing, whereas 35 subjects received oral hygiene instruction only. At baseline and 3 months after completion of periodontal treatment, clinical periodontal parameters were recorded. Serum levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and C-reactive protein (CRP), lipid profile markers and white blood cell count were assayed. Pearson's correlation analysis was applied to examine the correlation between the change of TNF- $\alpha$ , IL-6 and CRP levels and the change of periodontal parameters after non-surgical periodontal treatment.

**Results:** At baseline, there were no statistical differences in all clinical, biochemical parameters and cytokine levels between these two groups. Three months later in the treatment group, all clinical parameters improved significantly and the serum levels of TNF- $\alpha$ , IL-6, and CRP reduced significantly. Reduction of TNF- $\alpha$  was significantly positively correlated with the reduction of bleeding index and plaque index; reduction of IL-6 was significantly positively correlated with the reduction of clinical attachment loss; reduction of CRP was significantly positively correlated with the reduction of clinical attachment loss and plaque index.

**Conclusion:** Non-surgical periodontal therapy decreased serum TNF- $\alpha$ , IL-6 and CRP levels in CP subjects with stable CHD, which could help to reduce the inflammatory burden of stable coronary heart disease subjects.

**Key words:** periodontitis, coronary heart disease, non-surgical periodontal therapy, TNF- $\alpha$ , IL-6, CRP

Recent studies have shown that periodontitis may affect systemic inflammatory burden significantly, which would result in endothelial dysfunction, athero-

sclerotic plaque instability, dyslipidemia, and insulin resistance depending on the severity of periodontitis<sup>1-4</sup>. Some mechanisms that link local infection of periodontitis with systemic inflammatory burden were studied in experimental studies<sup>5-7</sup>. Periodontitis has become the focus of attention as a potential risk factor, which may mediate in the release coordination of cytokines, acute phase proteins and enzymes. Periodontal treatment is targeted to be a new way to reduce the systemic inflammation levels, in order to control the risk of systemic disease<sup>8-10</sup>.

Some studies have demonstrated that serum tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL-6) and

1 Department of Periodontology, Peking University School and Hospital of Stomatology, Beijing, P.R. China.

2 Department of Oral Therapy Center, Anzhen Hospital, Beijing, P.R.China.

3 Department of Cardiology, Anzhen Hospital, Beijing, P.R.China.

**Corresponding author:** Dr Xiang Ying OUYANG, Department of Periodontology, Peking University School and Hospital of Stomatology #22 Zhongguancun Nandajie, Haidian district, Beijing 100081, P.R. China. Tel: 86-10-82195372; Fax: 86-10-62173402; E-mail: kqouyangxy@bjmu.edu.cn

C-reactive protein (CRP) levels are elevated in severe chronic periodontitis subjects<sup>11-14</sup>, and most intervention studies reported that periodontal treatment decreased the levels of systemic inflammatory cytokines in systemic healthy subjects<sup>15-22</sup>. Till now, only few periodontal intervention studies were conducted in both coronary heart disease (CHD) and chronic periodontitis (CP) subjects<sup>23-27</sup>, and the change of serum levels of TNF- $\alpha$ , IL-6 and CRP was rarely reported. The observation regarding the effect of periodontal therapy on systemic TNF- $\alpha$ , IL-6 and CRP levels in both CHD and CP patients, will help us to further understand the connection between focal infection of periodontitis and systemic inflammatory status<sup>23-27</sup>.

Scientific evidence regarding the reduction of levels of inflammatory cytokines through periodontal therapy in both CHD and CP subjects remains inconclusive. Thus, the aim of this study was to examine whether non-surgical periodontal treatment could reduce serum levels of TNF- $\alpha$ , IL-6 and CRP to decrease the inflammatory burden of CHD subjects.

## Material and methods

### Subjects

This study was approved by the Institutional Review Board of Health Science Centre, Peking University, China. Seventy-five CHD stable subjects with chronic periodontitis were enrolled in the study. They were all > 40 years old and signed the informed consent form. CHD was diagnosed in the Department of Cardiology of Anzhen Hospital, Beijing. All subjects had stable CHD determined by fulfilling one of the following inclusion criteria: a previous history of myocardial infarction, or angioplasty surgery more than 6 months previously, or proven coronary or left main stem vessel obstruction by more than 50% as demonstrated by angiography. All subjects were examined blindly by a qualified periodontist using a standard Williams probe. Inclusion criteria for chronic periodontitis included possession of at least 16 teeth, with more than 30% of the teeth with probing depth (PD)  $\geq$  4 mm and clinical attachment loss (CAL)  $\geq$  3mm, and with alveolar bone loss > 30% of the root length as judged from panoramic radiographs. In addition, at least 2 teeth showed both PD  $\geq$  5 mm and CAL  $\geq$  3 mm and were distributed in different quadrants.

The exclusion criteria were as follows: acute myocardial infarction within the previous 6 months prior to recruitment; symptoms of unstable CHD; periodontal treatment within the previous 6 months; antibiotic treat-

ment within the previous 6 months; any surgery within the previous 6 months; a history of other systemic disease which affects the systemic inflammatory cytokine levels, such as diabetes, malignant tumour, respiratory disease, or liver disease; a history of hyperlipidaemia regularly taking anti-hyperlipidaemia medicine as statin.

### Study design

The subjects were enrolled consecutively in the Department of Cardiology of Anzhen Hospital during the years 2008 and 2009. After the baseline examinations, subjects were recruited to a treatment group or an untreated group according to their decision to start periodontal treatment within 1 week or not. This clinical trial was approved by the Ethical Committee of Peking University Health Science Centre, and all the subjects signed their informed consent.

Subjects in the treatment group started non-surgical periodontal therapy within one week. Non-surgical periodontal therapy included oral hygiene instruction (teaching brushing, flossing and usage of interdental brush), supragingival scaling and four sessions of quadrant root debridement (ultrasonic and hand instruments combined). No antibiotics were prescribed. Oral plaque control status was evaluated and prophylactic scaling was performed 6 weeks after treatment. Subjects were recalled 3 months after the end of the treatment.

Subjects in the untreated group only received oral hygiene education, and were recalled 3 months later and received periodontal treatment.

For the medication of stable CHD, it included expanding coronary drugs such as nitroglycerin, and heart rate lowering drugs, such as metoprolol, anti-coagulant aspirin or clopidogrel. For some hypertension patients, the medication included antihypertensive drugs such as Norvasc. The medication between the treatment and control group had no difference, the patients were informed not to change their habits of taking medications, smoking and diet over the course of the observation period, if they changed it, they needed to report the changes to the examiner.

### Laboratory testing

Fasting blood samples of the subjects were taken in the early morning from 8 to 10 o'clock, serum and plasma were isolated from blood samples of each patient. White blood cell counts (WBC) were performed in each patient. Total cholesterol (CHO), triglyceride (TG), high-density lipoprotein (HDL) and blood glucose lev-

**Table 1** General characteristics of subjects in treatment and control groups at baseline

| General characteristics                 | Treatment group<br>n = 40 | Control group<br>n = 35 |
|---|---------------------------|-------------------------|
| Age (years old)                         | 62.11 ± 9.30              | 62.48 ± 12.24           |
| Gender (male:female)                    | 33:7                      | 27:8                    |
| BMI (KG/m <sup>2</sup> )                | 25.25 ± 1.24              | 25.17 ± 1.18            |
| Percentage of smoking subjects (%)      | 11 (27.50%)               | 9 (25.71%)              |
| Percentage of hypertension subjects (%) | 3 (7.50%)                 | 3 (8.57%)               |

No significant difference was found for all these variables between the treatment and control groups.

els (GLU) were measured in the serum by enzymatic assays using a 7600-020 Automatic Analyzer (Hitachi). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula: LDL cholesterol = total cholesterol - [HDL + (TG/5)]. CRP was assayed by use of a BN-II BN100 automatic protein analysis system (Dade Behring, Marburg). Serum levels of IL-6 and TNF- $\alpha$  were determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits (R & D Systems) according to the manufacturer's instructions and DTX880 (Beckman Coulter) spectrophotometrically at optical density (OD) 450 nm.

### Statistical analysis

The data were presented as mean  $\pm$  standard deviation (SD). All clinical and biochemical parameters between baseline and 3 months were compared using Wilcoxon signed ranked matched pairs test within the treatment and control groups. Mann-Whitney U-tests were used to detect significant differences between the two groups at baseline and 3 months reexamination for all parameters. The Chi-square test was used to detect two group differences with respect to general characteristics, and the percentage change within a group. Pearson's correlation analysis was applied to examine the correlation between the change of TNF- $\alpha$ , IL-6 and CRP levels and the change of periodontal parameters after non-surgical periodontal treatment, and the correlation between the changes of them with their baseline levels.

### Results

The periodontal treatment group included 40 patients – 33 men and 7 women with a mean age of 62.11  $\pm$  9.30

years old. The untreated group had 35 patients – 27 men and 8 women with a mean age of 62.48  $\pm$  12.24 years old. No subjects withdrew from this study, or required emergency treatment for CHD-related problems. The habit of medication, smoking and diet did not change over the course of the observation period. The examination of all clinical parameters was conducted by one periodontist, whose intraexamination reliability of periodontal parameters was 80 to 90%.

### Baseline status of subjects

No significant difference was found between the treatment and control group for the general characteristics of subjects (age, gender, body mass index, smoking status, and percentage of hypertension) (Table 1).

No significant difference was found for all periodontal parameters (Table 2) and biochemical parameters (including serum glucose levels, lipid profile, and WBC counts) (Table 3) at baseline between treatment and control group. Biochemical indices were normal except serum TG levels (1.61  $\pm$  0.78 mmol/L in treatment group, 1.82  $\pm$  1.20 mmol/L in control group, normal range 0.25-1.24 mmol/L).

### The effect of periodontal therapy on periodontal status

At 3 months after treatment, all clinical parameters, including PD, CAL, plaque index (PLI), bleeding index (BI) and the percentage of sites that had a PD  $\geq$  5mm, improved significantly ( $P < 0.001$ ) in the treatment group, but not in the control group. From baseline to 3 months, the changes in all clinical parameters in the treatment group were statistically significant compared with that of the control group ( $P < 0.001$ ) (Table 2).



**Table 2** Periodontal clinical parameters in treatment and control group

| Clinical parameters | Treatment group (n = 40)<br>Mean ± SD |             |               |        | Control group (n = 35)<br>Mean ± SD |              |              |       |
|---------------------|---------------------------------------|-------------|---------------|--------|-------------------------------------|--------------|--------------|-------|
|                     | Baseline                              | 3 months    | Δ             | P      | Baseline                            | 3 months     | Δ            | P     |
| PD (mm)             | 3.95 ± 0.13                           | 2.94 ± 0.20 | 1.01 ± 0.20*  | <0.001 | 3.92 ± 0.23                         | 3.93 ± 0.23  | -0.00 ± 0.05 | 0.621 |
| CAL (mm)            | 3.07 ± 0.46                           | 2.45 ± 0.47 | 0.61 ± 0.35*  | <0.001 | 2.97 ± 0.46                         | 2.99 ± 0.45  | -0.03 ± 0.07 | 0.070 |
| PLI                 | 1.63 ± 0.23                           | 1.09 ± 0.15 | 0.53 ± 0.19*  | <0.001 | 1.61 ± 0.16                         | 1.60 ± 0.15  | 0.00 ± 0.06  | 0.487 |
| BI                  | 2.71 ± 0.43                           | 1.53 ± 0.17 | 1.17 ± 0.35*  | <0.001 | 2.50 ± 0.35                         | 2.49 ± 0.29  | 0.01 ± 0.11  | 0.580 |
| PD ≥ 5 mm (%)       | 20.20 ± 6.87                          | 3.32 ± 1.94 | 17.87 ± 6.52* | <0.001 | 21.74 ± 7.28                        | 22.14 ± 3.38 | -0.36 ± 1.26 | 0.117 |

Δ: Change between baseline and 3 months within a group

P: P value for change between baseline and 3 months within a group (Wilcoxon's signed-rank test).

\*: P < 0.001 when compared with control group 3 months after non-surgical periodontal therapy (Mann-Whitney U-test).

**Table 3** Biochemical parameters and WBC counts in treatment and control groups

|              | Treatment group (n = 40)<br>Mean ± SD |             |              |       |             |             |              |       |
|--------------|---------------------------------------|-------------|--------------|-------|-------------|-------------|--------------|-------|
|              | Baseline                              | 3 months    | Δ            | P     | Baseline    | 3 months    | Δ            | P     |
| GLU (mmol/L) | 5.73 ± 1.18                           | 5.51 ± 0.81 | 0.22 ± 0.86  | 0.114 | 6.01 ± 1.57 | 6.15 ± 1.29 | -0.14 ± 0.86 | 0.378 |
| CHO (mmol/L) | 4.24 ± 0.84                           | 3.88 ± 0.84 | 0.35 ± 1.07  | 0.058 | 4.58 ± 1.00 | 4.63 ± 1.00 | -0.05 ± 0.65 | 0.696 |
| TG (mmol/L)  | 1.61 ± 0.78                           | 1.53 ± 0.68 | 0.08 ± 0.45  | 0.284 | 1.82 ± 1.20 | 1.68 ± 0.96 | 0.13 ± 0.72  | 0.315 |
| HDL (mmol/L) | 1.01 ± 0.18                           | 1.01 ± 0.22 | 0.00 ± 0.19  | 0.944 | 1.07 ± 0.24 | 1.11 ± 0.27 | -0.04 ± 0.20 | 0.279 |
| LDL (mmol/L) | 2.70 ± 0.78                           | 2.33 ± 0.84 | 0.38 ± 1.00# | 0.032 | 2.84 ± 0.85 | 2.98 ± 0.87 | -0.14 ± 0.58 | 0.207 |
| WBC (g/L)    | 7.56 ± 2.66                           | 7.28 ± 1.91 | 0.28 ± 1.58  | 0.281 | 6.75 ± 1.74 | 6.67 ± 1.79 | 0.09 ± 1.25  | 0.718 |

Δ: Change between baseline and 3 months within a group.

P: P value for change between baseline and 3 months within a group (Wilcoxon's signed-rank test).

#: P = 0.013 when compared with control group 3 months after non-surgical periodontal therapy (Mann-Whitney U-test).

*The effect of periodontal therapy on WBC counts, serum lipid and glucose levels*

As shown in Table 3, at 3 months, serum levels of LDL in the treatment group declined significantly (from 2.70 ± 0.78 mmol/L to 2.33 ± 0.84 mmol/L; P = 0.032), but no changes in the control group were observed. The level of GLU, CHO, TG, HDL and WBC did not change significantly from baseline to the 3-month reassessment within both groups (Table 3).

From baseline to 3 months, the changes in LDL level (ΔLDL) in the treatment group were statistically significant compared with that of the control group (P=0.013) (Table 3).

*The effect of periodontal therapy on TNF-α, IL-6 and CRP levels*

At baseline, no significant difference was found for serum TNF-α, IL-6 and CRP levels between the treat-

**Table 4** Serum levels of TNF- $\alpha$ , IL-6 and CRP in treatment and control group

|                       | Treatment group (n = 40)<br>Mean $\pm$ SD |                   |                    |       | Control group (n = 35)<br>Mean $\pm$ SD |                   |                   |       |
|-----------------------|---|-------------------|--------------------|-------|---|-------------------|-------------------|-------|
|                       | Baseline                                  | 3 months          | $\Delta$           | P     | Baseline                                | 3 months          | $\Delta$          | P     |
| TNF- $\alpha$ (pg/mL) | 39.88 $\pm$ 33.83                         | 28.99 $\pm$ 16.56 | 12.20 $\pm$ 35.59# | 0.048 | 49.61 $\pm$ 69.60                       | 50.64 $\pm$ 86.33 | -8.51 $\pm$ 43.71 | 0.917 |
| IL-6 (pg/mL)          | 38.61 $\pm$ 21.87                         | 31.40 $\pm$ 20.32 | 7.58 $\pm$ 22.20#  | 0.049 | 39.12 $\pm$ 24.31                       | 42.56 $\pm$ 23.81 | -8.16 $\pm$ 26.66 | 0.111 |
| CRP (mg/L)            | 2.61 $\pm$ 3.16                           | 2.06 $\pm$ 2.54   | 0.69 $\pm$ 1.29#   | 0.007 | 2.96 $\pm$ 3.55                         | 2.94 $\pm$ 3.62   | 0.06 $\pm$ 1.08   | 0.741 |

$\Delta$ : Change between baseline and 3 months within a group.

P: P value for change between baseline and 3 months within a group (Wilcoxon's signed-rank test).

#: P = 0.047, 0.015, 0.032 for TNF- $\alpha$ , IL-6 and CRP respectively when compared with control group 3 months after non-surgical periodontal therapy (Mann-Whitney U-test).

**Table 5** Correlation between change of cytokine levels and change of periodontal parameters as well as their baseline level

| Correlation coefficient | $\Delta$ TNF- $\alpha$ |       | $\Delta$ IL-6 |       | $\Delta$ CRP |       |
|-------------------------|------------------------|-------|---------------|-------|--------------|-------|
|                         | r                      | P     | r             | P     | r            | P     |
| $\Delta$ PD             | 0.186                  | 0.100 | 0.220         | 0.051 | 0.180        | 0.098 |
| $\Delta$ CAL            | 0.198                  | 0.081 | 0.238         | 0.035 | 0.215        | 0.047 |
| $\Delta$ BI             | 0.222                  | 0.049 | 0.208         | 0.066 | 0.171        | 0.115 |
| $\Delta$ PLI            | 0.255                  | 0.023 | 0.204         | 0.071 | 0.219        | 0.043 |
| Baseline TNF- $\alpha$  | 0.169                  | 0.137 | 0.045         | 0.091 | 0.110        | 0.335 |
| Baseline IL-6           | 0.023                  | 0.838 | 0.606         | 0.000 | 0.010        | 0.928 |
| Baseline CRP            | 0.086                  | 0.449 | 0.152         | 0.181 | 0.346        | 0.001 |

$\Delta$ TNF- $\alpha$ ,  $\Delta$ IL-6,  $\Delta$ CRP: Change of TNF- $\alpha$ , IL-6 and CRP level between baseline and 3 months after non-surgical periodontal therapy in treatment group;  $\Delta$ PD,  $\Delta$ CAL,  $\Delta$ BI,  $\Delta$ PLI: Change of PD, CAL, BI and PLI between baseline and 3 months after non-surgical periodontal therapy in treatment group.

ment and control groups. At 3 months, serum TNF- $\alpha$ , IL-6 and CRP levels were statistically significantly reduced in the treatment group ( $P = 0.048, 0.049, 0.007$  respectively), but not in the control group (Table 4).

From baseline to 3 months, the changes in TNF- $\alpha$  ( $\Delta$ TNF- $\alpha$ ), IL-6 ( $\Delta$ IL-6), and CRP ( $\Delta$ CRP) levels in the treatment group were statistically significant compared with that of the control group ( $P = 0.047, 0.015, 0.032$ , respectively) (Table 4).

#### Pearson's correlation analysis

Pearson's correlation analysis showed that there was a significant positive correlation between the reductions of cytokine levels with the reduction of periodontal indices after non-surgical treatment in the treatment group.  $\Delta$ TNF- $\alpha$  was significantly positively correlated with  $\Delta$ BI and  $\Delta$ PLI ( $r = 0.222, 0.255, P = 0.049, 0.023$ , respectively);  $\Delta$ IL-6 was significantly positively correlated with  $\Delta$ CAL ( $r = 0.238, P = 0.035$ );  $\Delta$ CRP was significantly pos-

itively correlated with  $\Delta$ CAL and  $\Delta$ PLI ( $r=0.215, 0.219, P=0.047, 0.043$  respectively). The similar positive correlation profiles were also observed between  $\Delta$ IL-6 and baseline IL-6 level ( $r=0.606, P=0.000$ ), between  $\Delta$ CRP and baseline CRP level ( $r=0.346, P=0.001$ ) (Table 5).

## Discussion

Local infection is considered to be a contributor to the systemic inflammation burden. In the present study, non-surgical periodontal treatment significantly reduced serum levels of TNF- $\alpha$ , IL-6 and CRP in 40 subjects with both CP and CHD. Pearson's correlation analysis showed that the reduction of TNF- $\alpha$ , IL-6 and CRP levels was significantly positively correlated with the reduction of periodontal clinical parameters after non-surgical periodontal therapy. At baseline, there is no difference between treatment and control group with respect to demographic, medical history, periodontal parameters and inflammatory cytokines level; however, the periodontal parameters and cytokines level in treatment group declined significantly at the end of the trial. Our results indicated that local periodontal treatment could reduce the systemic cytokines level in CP subjects with CHD.

TNF- $\alpha$  is the important cytokine that is secreted by leukocytes, macrophages and lymphocytes in the local lesion of periodontitis. The possible mechanisms that connect focal infection in periodontitis with systemic cytokine levels might be that the TNF- $\alpha$  stimulated the expression of IL-6, which consequently augmented the CRP gene expression in the liver<sup>5-7</sup>. In this study, the systemic reduction of TNF- $\alpha$  level was positively correlated with reduction of BI and PLI, BI and PLI are important inflammatory indices of periodontitis, which might be part of the proof of the mechanism that links the focal infection with systemic cytokine levels.

The reduction of serum level of IL-6 and CRP after non-surgical treatment in this study was consistent with the previous studies. Research by Montebugnoli et al observed CRP, leucocytes, and fibrinogen levels in 18 male subjects with proven CHD; they found that CRP levels reduced 3 months after periodontal treatment<sup>23</sup>. Tüter et al randomly distributed 36 patients with both CP and CHD into two groups (Placebo or SDD; 6 weeks) and both received two regimens of scaling and root planing (SRP), it was reported the significant improvement in serum levels of CRP, apolipoprotein-

A (APO-A), HDL post-treatment in both groups<sup>24</sup>. Higashi et al found periodontal therapy reduced serum concentrations of CRP and IL-6 in CP and CHD subjects<sup>25</sup>. Hussain et al reported that CRP, fibrinogen, and WBC counts were reduced significantly after periodontal therapy in 27 CHD patients<sup>26</sup>. In our study, the similar results were achieved in the reduction of serum CRP and IL-6 levels, we also found the reduction of IL-6 and CRP levels was positively correlated with reduction or periodontal parameters after periodontal therapy.

In the present study, the serum LDL level in the treatment group was significantly reduced after the treatment, and this reduction was significantly different from the control group. Montebugnoli et al reported the declined level of ox-LDL<sup>23</sup> and Tuter et al reported the increased level of HDL in subjects with CHD and CP after periodontal treatment<sup>24</sup>. Some studies reported certain lipid markers improved after periodontal treatment in subjects with CP and hyperlipidemia<sup>28,29</sup>, some not<sup>25</sup>. Lipid profile can be influenced by many confounding factors, which may explain the different results reported by different studies.

More men enrolled in this study than women, it may be relevant to the fact the prevalence of periodontitis and CHD in men is higher than that in women. Though it was not strictly randomised design, there were no differences in the parameters between treatment and control group at baseline, but at 3 months later, significant differences between the two groups existed in periodontal parameters and inflammatory biomarkers levels. In addition, the subjects in this study regularly take the anticoagulant aspirin or clopidogrel, which exerts inhibitory effect in inflammatory cytokines levels. However, Person's correlation analysis showed that the reduction of TNF- $\alpha$ , IL-6 and CRP level was significantly positively correlated with the reduction of periodontal parameters. It might be deduced that the significant difference in inflammatory cytokine level between the two groups at the end of the study resulted from periodontal intervention.

In summary, the present study conducted in 75 Chinese subjects with both CHD and CP showed that periodontal treatment significantly reduced serum TNF- $\alpha$ , IL-6 and CRP levels. Thus, periodontal therapy might provide an effective approach to decrease the systemic cytokine levels. Future well-designed studies with a larger population and a longer duration should be performed to validate these findings.

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### Format of Papers

#### Preparation of manuscripts

The manuscript should be written clearly and concisely and be double-spaced on 21 x 29 cm white paper with at least 2.5 cm margin all around. All pages should be numbered, beginning from section of title page, and followed by abstract, introduction, materials and methods, results, discussion, acknowledgements, references, figure legends and figures/tables. Non-standard abbreviations should be defined when first used in the text. Use a standard font such as Times New Roman or Arial to avoid misrepresentation of your data on different computers that do not have the unusual or foreign language fonts. For units, the Journal recommends the use of the International System of Units (SI Units). For authors whose native language is not English, the Journal strongly recommends improving the English in the manuscript by consulting an English-speaking scientist before submission.

**Title page** should include: full title (a brief declarative statement of the major findings of the research), full names of authors, professional affiliations and complete postal address, telephone and fax number, and email address of the corresponding author. If the work was supported by a grant, indicate the name of the supporting organisation and the grant number.

**Abstract and keywords:** 250 words presented in a concise form and including the purpose, general methods, findings, and conclusions of the research described in the paper. A list of 5 keywords or short phrases (a few words per phrase) suitable for indexing should be typed at the bottom of the abstract page. Avoid vague or overly general terms. If necessary, the keywords will be adjusted to the standards of the Journal by the editors without consulting the authors.

**Introduction:** should begin with a brief introduction of background related to the research and should be as concise as possible. The rationale of the study should be stated.

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1. Sorensen JA, Engleman MT, Torres TJ, Avera SP. Shear bond strength of composite resin to porcelain. *Int J Prosthodont* 1991;4:17-23.
2. Renner RP, Boucher LJ. Removable Particle Dentures. Chicago: Quintessence, 1987:24-30.
3. White GE, Johson A van Noort R, Northeast SE, Winstanley B. The quality of cast metal ceramic crowns made for the NHS [abstract 48]. *J Dent Res* 1990;69(special issue):960.
4. Jones DW. The strength and strengthening mechanisms of dental ceramics. In: McLean JW (ed). *Dental ceramics: Proceedings of the First International Symposium on Ceramics*. Chicago: Quintessence, 1983:83-41.
5. Rosenstiel S. *The Marginal Reproduction of Two Elasto meric Impression Materials* [Master's thesis]. Indianapolis: Indiana University, 1997.

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