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## Periodontitis in a High-altitude Hypoxic Environment Through Rat Model Simulation

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### ABSTRACT

The morbidity of periodontitis is considered significantly higher in plateau areas than in other areas. In this study, we aimed to establish a rat model that simulates hypoxic periodontitis and investigate the pathogenesis of periodontitis in plateau hypoxic areas. Sprague-Dawley (SD) rats (n = 80) were randomly divided into hypoxic control group, hypoxic experimental group (HE group), normoxic control group and normoxic experimental group (NE group). We discussed the correlation between clinical periodontal parameters and cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and interleukin-8 (IL-8), in Gingival Crevicular Fluid (GCF). Results showed that the SD rat model successfully simulated human periodontitis. TNF- $\alpha$  and PGE<sub>2</sub> levels in GCF of the HE group were significantly higher than those of the NE group (p<0.01). Attachment loss, plaque index and bleeding index showed a positive correlation with TNF- $\alpha$  and PGE<sub>2</sub>. However, IL-8 level in the HE group was lower than that in the control groups (p<0.01). A negative correlation was observed between IL-8 and the clinical periodontal parameters (p<0.01). The hypoxic environment may influence the expression and the secretion of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in periodontal tissues. TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 were significantly related with clinical periodontal parameters in hypoxic environments.

**Key words:** Periodontitis, high altitude hypoxia environment, clinical periodontal parameter, cytokines

### INTRODUCTION

Periodontitis is a set of inflammatory diseases that affect the tissues that surround and support the teeth. Periodontitis which involves gingival inflammation, attachment loss, periodontal pocket and progressive alveolar bone loss, is frequently encountered in the oral cavity and is the primary cause of tooth loss in adults (Petersen *et al.*, 2005). Accumulated evidence has revealed that periodontitis is directly correlated to plaque microorganisms and associated specific derivatives that stimulate a local inflammatory reaction and indirectly trigger an overly aggressive immune response of the host against these microorganisms. Researchers also revealed that tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and interleukin-8 (IL-8), among various kinds of these inflammatory mediator cytokines, are closely related to the occurrence and development of periodontitis (Huang *et al.*, 2001; Garlet *et al.*, 2005; Liu *et al.*, 2007; Asikainen *et al.*, 2010; Ebersole *et al.*, 2010; Andia *et al.*, 2011).

Several epidemiological surveys have indicated that the morbidity of periodontitis is significantly higher in plateau areas than in other areas; thus, morbidity also increases at increasing altitudes and with increasing age (Garlet *et al.*, 2006). Regardless of different host factors and lifestyles of individuals in these areas, the hypoxic environment significantly affects the pathogenesis of periodontitis. Studies have shown that high altitude or hypoxia can influence systemic and periodontal microenvironment, thereby exacerbating periodontal inflammation. This study was designed to investigate the pathogenesis of periodontitis in a population that lives in a high-altitude environment.

We also aimed to create a model by using Sprague-Dawley (SD) rats to simulate hypoxic periodontitis and thus, determine whether or not hypoxic environments affect clinical and histopathological characteristics as well as specific cytokines of periodontitis. Spearman's method was used to analyze the correlation between the clinical periodontal parameters and the density of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in Gingival Crevicular Fluid (GCF).

## MATERIALS AND METHODS

**Animal models:** Clean and healthy SD rats (n = 80; 40 males and 40 females) were randomly distributed into four groups [20 rats per group; hypoxic control group (HC group), hypoxic experimental group (HE group), normoxic control group (NC group) and normoxic experimental group (NE group)] after they were adaptively fed for two weeks. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Second Affiliated Hospital of Third Military Medical University. The rats were bred and treated separately. The animal model with periodontitis was built according to Fischer's method (Han *et al.*, 2006). To induce periodontitis, an orthodontic steel ligature (diameter = 0.2 mm) was placed around the right maxillary first molar at the sub-marginal position of each SD rat from the two experimental groups. The result is shown in Fig. 1. The rats in the experimental groups were fed with high-sugar food that contains 100 g L<sup>-1</sup> of glucose, 60 g L<sup>-1</sup> of sucrose and 30 g of milk powder (Breivik *et al.*, 2000; Muia *et al.*,

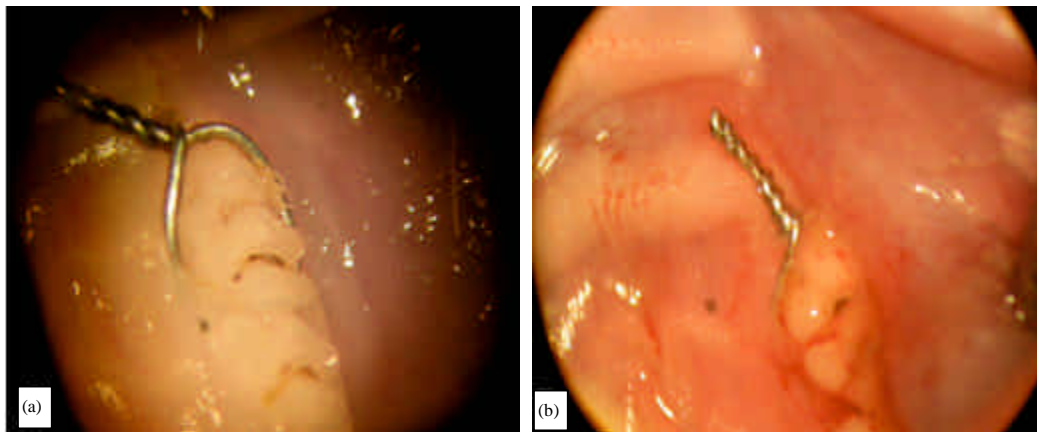


Fig. 1(a-b): (a) The Sprague-Dawley (SD) rats' model of periodontitis. An orthodontic steel ligature (0.2 mm Dia.) was placed around the right maxillary first molar at the submargin and (b) The SD rats model of periodontitis 8 weeks later

Table 1: Experimental rat groups

Group	No.	Diet	Ligation	Environment	Hypoxic time	Prednisolone
NC	20	Rat food	No	Normoxia	No	No
HC	20	Rat food	No	Hypoxia	23 h day <sup>-1</sup>	No
NE	20	High sugar	Yes	Normoxia	No	I.M.
HE	20	High sugar	Yes	Hypoxia	23 h day <sup>-1</sup>	I.M.

NC: Normoxic control group, HC: Hypoxic control group, NE: Normoxic experimental group, HE: Hypoxic experimental group, I.M.: Intramuscular injection

2006; Xu and Wei, 2006). HC and HE groups were then bred in hypobaric chambers at an atmospheric pressure of 54.047 kPa (oxygen concentration 10-11%) to simulate the hypoxic environment at an altitude of 5000 m. The two hypoxic groups were placed in the hypobaric chambers for 23 h except one treatment which was subjected to standard pressure for 1 h. The treatment methods for the four groups are listed in Table 1.

**Detection of clinical periodontal parameters:** The experimental animals were anesthetized with 3% pentobarbital sodium (40 mg kg<sup>-1</sup>) and the periodontal parameters of SD rats were detected after eight weeks.

**Bleeding Index (BI):** The tip of the periodontal probe was placed at 1 mm under the gingival margin and slid slightly. The bleeding degree was detected and scored according to the following scale (0) Healthy periodontium, (1) Mild periodontal inflammation (slightly changed color of the gingiva, mildly dropsy and no bleeding upon probing), (2) Moderate periodontal inflammation (reddened gingiva, significantly dropsy and bleeding upon probing) and (3) Severe periodontal inflammation (gingiva with apparent redness and swelling, ulceration and spontaneous bleeding).

**Plaque Index (PLI):** The index was scored according to the thickness and the quantity of the dental plaque by slightly scratching the tooth with a probe (0) No plaque, (1) A plaque film that adheres to the free gingival margin and the adjacent area of the tooth, (2) Moderate plaque accumulation within the gingival pocket or the tooth surface and the gingival margin and (3) Abundance of plaque within the gingival pocket and/or the tooth and the gingival margin.

**Probing Depth (PD):** The periodontal pocket of the target tooth was investigated using a dental graduated probe. Mesio Buccal, buccal central and distobuccal points were then selected for measurements and the average values were recorded.

**Attachment Loss (AL):** After PD was measured, the position of the Cement-Enamel Junction (CEJ) was detected by the probe. The distance between CEJ and the gingival margin was measured. AL was obtained by subtracting it from PD.

GCF quantity was detected according to a previous study (Zhao and Deng, 2007). The right maxillary first molar was dried and isolated by using a sterile cotton ball to prevent saliva contamination. Three pre-weighed Whatman paper strips (2×20 mm) were separately plugged into the periodontal pocket at three different positions: mesio Buccal, buccal central and distobuccal. After 30 sec, the paper strips were removed and preserved in a pre-weighed centrifuge tube. The GCF weight was calculated by subtracting the original weight from the total weight of the centrifuge tube and the paper strips which were dipped in GCF. GCF was then preserved at -70°C.

Histopathological observation was subsequently performed. After the rats were bred and observed for eight weeks, the SD rats in the four groups were sacrificed by cervical dislocation. The predetermined tooth (right maxillary first molar) and its surrounding tissues of each rat were immediately removed and preserved for further observation.

Pathological sections were obtained according to the following steps:

- Paraformaldehyde fixation
- Decalcification and dehydration
- Paraffin embedding and sectioning (4 to 6 slices with a thickness of 5  $\mu\text{m}$  for each sample)
- Hematoxylin-eosin (HE) staining
- Resinous cover slipping. The infiltrated areas of the inflammatory cells in epithelial and connective tissues, the structure and orientation of periodontal fibers, the presence of osteoclasts and alveolar bone resorption were observed by optical microscopy

TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 expression in periodontal tissues was also determined. Conventional Streptavidin-biotin Complex (SABC) method was used to cut the tissues into sections. Rabbit TNF- $\alpha$  monoclonal antibody, rabbit PGE<sub>2</sub> monoclonal antibody and rabbit IL-8 monoclonal antibody were used in the SABC method.

TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 concentrations in GCF were also determined. The unfrozen paper strips dipped in GCF were added into 0.5 mL of phosphate buffer solution (pH 7.4), steeped for 30 min and oscillated for 10 min at room temperature. After the resulting mixture was centrifuged at low temperature for 20 min, the supernatant was drawn and preserved in a clean numbered Eppendorf tube at -20°C. The TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 concentrations in GCF were detected by enzyme-linked immunosorbent assay (Teles *et al.*, 2009). The Optical Density (OD) was determined by using the Tecan-Sunrise microplate reader (Switzerland). Based on the standard curves plotted on Curve Expert 1.3, the densities of these cytokines were calculated (Barer *et al.*, 1983; Stelzner *et al.*, 1988; Howell *et al.*, 2003).

**Statistical analysis:** Statistical analysis was performed on SPSS 10.0. Data were presented as mean $\pm$ standard deviation and analyzed by one-way ANOVA. If variance was equal, t-test was used for analysis. Otherwise, Tamhane's T<sup>2</sup> test was conducted. Spearman's method was used for correlation analysis between periodontal clinical parameters and densities of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in GCF. Differences at  $p < 0.05$  were considered statistically significant.

## RESULTS AND DISCUSSION

**Clinical periodontal parameters:** After the SD rats were bred for eight weeks, the SD rats exhibited periodontal tissue erosion and atrophy. A large amount of food debris was obtained around the target experimental tooth. Gingival bleeding by touching or spontaneous bleeding occurred. Periodontal PLI and PD as well as the height of the alveolar crest changed significantly (Fig. 1). A significant difference was also observed between the HE group and the other groups in terms of three clinical periodontal parameters: BI, PD and PLI ( $p < 0.01$ ; Table 2). The mean AL in the HE group was 3.14 mm which was higher than that in the NE group. The amount of GCF in the HE group was 0.1907 mg which was higher than that in NC (0.1321 mg), HC (0.1404 mg) and NE (0.1795 mg) groups (Fig. 2).

GCF contains substances that come mainly from the serum and other constituents from the epithelial cells, leukocytes, as well as subgingival and supragingival plaque of hosts (Lamster and Ahlo, 2007). GCF is considered as a microenvironment where bacteria and the host interact because

Table 2: Clinical periodontal parameters of four groups

Group	BI	PD (mm <sup>-1</sup> )	PLI	AL (mm <sup>-1</sup> )
NC	0.63±0.08	0.21±0.03	0.62±0.08	0.85±0.06
HC	0.74±0.11	0.33±0.04	0.64±0.09	0.91±0.08
NE	2.12±0.14 <sup>a</sup>	1.45±0.12 <sup>a</sup>	1.28±0.11 <sup>a</sup>	3.10±0.15 <sup>a</sup>
HE	2.64±0.20 <sup>bd</sup>	1.83±0.20 <sup>bc</sup>	1.65±0.17 <sup>bc</sup>	3.14±0.12 <sup>bc</sup>

<sup>a</sup>p<0.05 NC group compared with NE group, <sup>b</sup>p<0.01, HE group compared with HC group, <sup>c</sup>p<0.01, HE Group compared with NE group. <sup>d</sup>p<0.05 HC group compared with NC group, NC: Normoxic control, HC: Hypoxic control, NE: Normoxic experimental, HE: Hypoxic experimental, BI: Bleeding index, PD: Probing depth, PLI: Plaque index, AL: Attachment loss

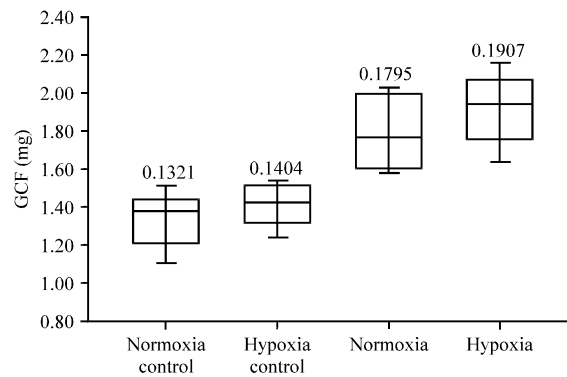


Fig. 2: The quantity (mg<sup>-1</sup>) of SD rats' right maxillary molar Gingival Crevicular Fluid (GCF) from four groups HC: Hypoxic control, HE: Hypoxic Experimental, NC: Normoxic Control and NE: Normoxic Experimental

of its unique location and complex composition. Previous reports demonstrated that the GCF outflow is proportional to the degree of inflammation (Lamster and Ahlo, 2007; Yamasaki *et al.*, 2009). In our study, similar experimental data were obtained.

**Histopathological observation:** In HC and NC groups, the epithelial tissues that were attached to the teeth had no pathological change (Fig. 3). The periodontal fibers and the cementoblasts were neatly organized. The alveolar crest had no absorption and the alveolar bone was not damaged. The gingival epithelium remained almost intact and the gingival lamina propria was infiltrated by a few neutrophils.

In the NE group, the epithelial tissues eroded and proliferated in a mesh-like fashion toward the connective tissues (Fig. 3). The infiltration of a large number of inflammatory cells and osteoclasts at the alveolar crest was observed. The periodontal membrane space widened but the height of the alveolar crest had no evident change.

In the HE group, we observed that reticular degeneration occurred in the epithelial tissues. The collagen fibers degenerated and swelled. The periodontal fibers exhibited a disorderly pattern, in which the periodontal membrane space widened and the inflammatory cells showed a significant infiltration. The alveolar crest and the proper alveolar bones were absorbed and damaged. Numerous osteoclasts were located in the alveolar absorption lacunae (Fig. 3).

The visual observation on the variation of periodontal tissues and clinical periodontal parameters, including BI, AL, PLI and PD in SD rats indicated that the rats in the HE group had significantly more serious periodontitis than in the other three groups. This result is consistent with that in previous studies on humans who live on plateaus (Arnett *et al.*, 2003; Liu *et al.*, 2007). The

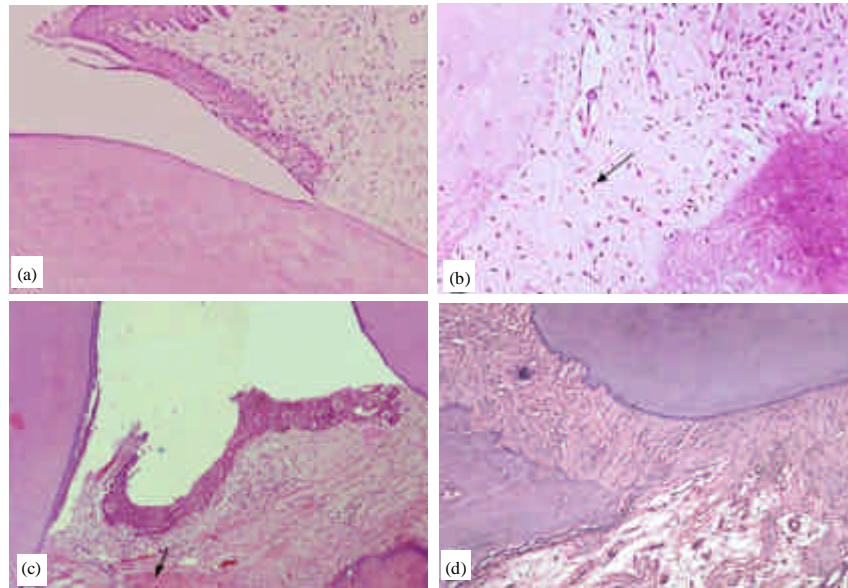


Fig. 3(a-d): The periodontal pathological tissue section of the four groups. (a) Hypoxic Control (HC) group, (b) Normoxic Control (NC) group, the black arrow indicates the neutrophils that infiltrated the gingival lamina propria, (c) Hypoxic Experimental (HE) group, the black arrow indicates the alveolar absorption lacunae where osteoclasts were located and (d) Normoxic Experimental (NE) group

relatively high morbidity of periodontitis in hypoxic environments is possibly caused by two factors. (1) Less amount of oxygen in hypoxic environments easily promotes the growth and the reproduction of periodontitic anaerobes and (2) The damage from plaque is more severe and faster than that under common conditions. Hypoxia may also slow down the blood flow.

Frequent symptoms of patients with chronic mountain sickness include sleep disorders, headaches, dizziness, tinnitus, paresthesia, physical weakness and mental fatigue (Penaloza and Arias-Stella, 2007). These symptoms are possibly the result of hemorheological changes caused by hypobaric hypoxia. Normal cell metabolism in periodontal tissues cannot proceed efficiently because of microcirculation stagnation and cyanosis. However, HE staining indicated that periodontitis in the simulated hypoxic environment on the plateau was similar to that in flatlands; in particular, no gingival connective tissues were cyanotic. In this case, further studies are necessary to determine whether or not cyanosis aggravates periodontitis. The increase in anaerobic metabolism can increase the amount of acidic and toxic substances which may initiate or aggravate periodontitis (Hofbauer *et al.*, 1999; Penaloza and Arias-Stella, 2007).

**Cytokine expression:** Figure 4 shows that the positive-stained positions of TNF- $\alpha$  and PGE<sub>2</sub> were mainly located in the cytoplasm of osteoclasts and parodontium fibroblasts. The stained position of IL-8 was located in the cytoplasm of neutrophil granulocytes (Fig. 4). The cytokine expression in different groups is listed in Table 3. Statistical analysis revealed that cytokines were expressed higher in the HC group than in the NC group ( $p < 0.05$ ). Similar results were obtained: HE group versus NE group ( $p < 0.05$ ); HE group versus HC group ( $p < 0.05$ ) and NE group versus NC group ( $p < 0.05$ ).

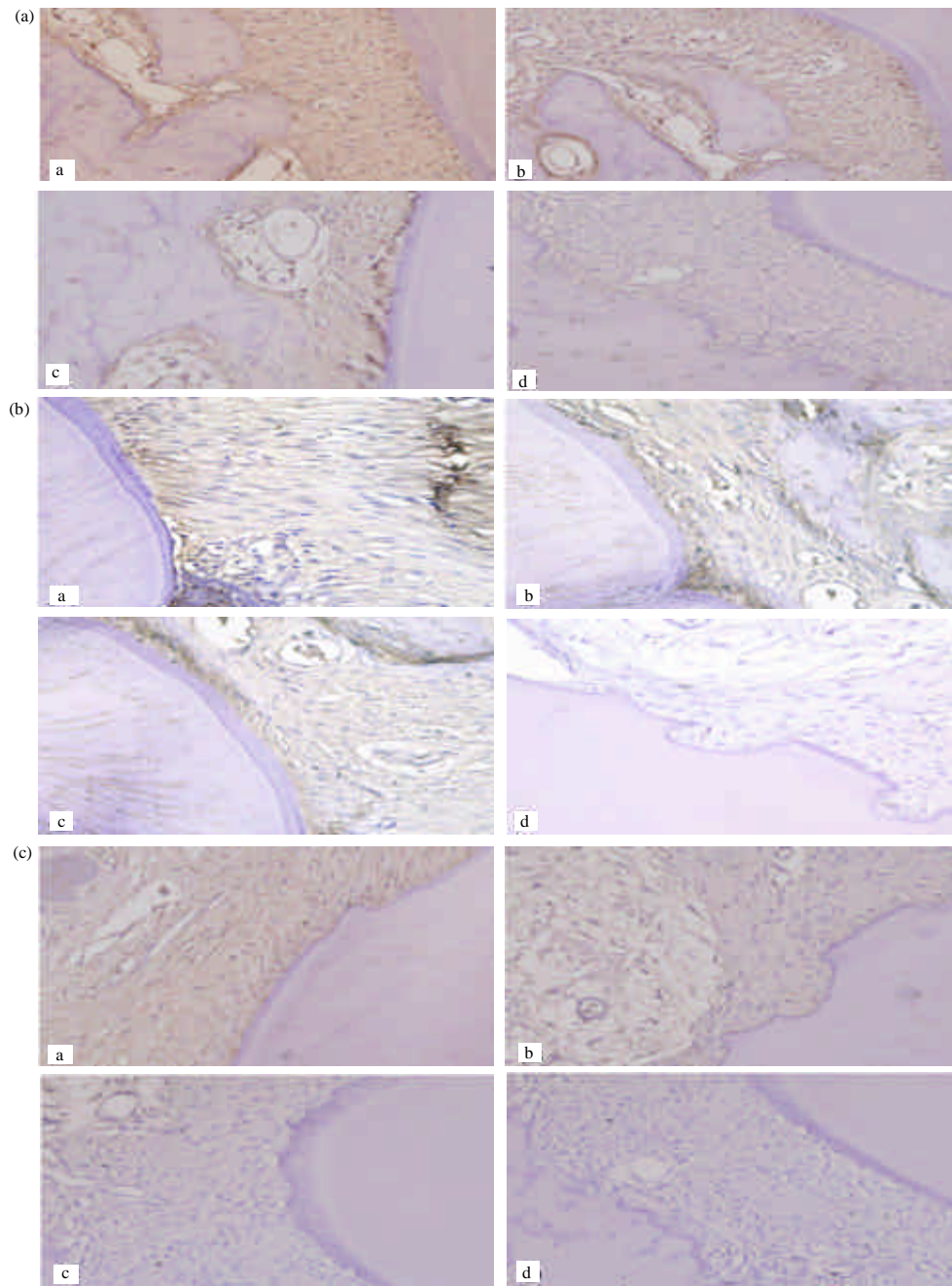


Fig. 4(a-c): The expression of TNF- $\alpha$ , PGE<sub>2</sub>, IL-8 in SD rats' periodontic tissues. TNF- $\alpha$ : (a) The expression of TNF- $\alpha$  in Sprague-Dawley (SD) rats' periodontic tissues (conventional streptavidin biotin complex (SABC)  $\times 200$ ). a: Hypoxic Experimental (HE) group, b: Normoxic Experimental (NE) group, c: Hypoxic Control (HC) group and d: Normoxic Control (NC) group PGE<sub>2</sub>: (b) The expression of PGE<sub>2</sub> in rats' periodontic tissues (SABC $\times 200$ ). a: HE group, b: NE group, c: HC group and d: NC group and (c) Expression of IL-8: The expression of IL-8 in SD rats' periodontic tissues (SABC $\times 200$ ). a: HE group, b: NE group, c: HC group and d: NC group



Table 3: Expression of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in periodontal tissues of four different groups

Groups	TNF- $\alpha$ positive				PGE <sub>2</sub> positive				IL-8 positive			
	+	++	+++	Rate (%)	+	++	+++	Rate (%)	+	++	+++	Rate (%)
NC	0	0	0	0	0	0	0	0	0	0	0	0
HC	6	2	1	45 <sup>a</sup>	5	2	1	40 <sup>a</sup>	2	2	2	40 <sup>a</sup>
NE	3	4	4	55 <sup>be</sup>	2	4	4	50 <sup>be</sup>	1	2	3	50 <sup>be</sup>
HE	2	5	6	65 <sup>d</sup>	3	4	6	65 <sup>d</sup>	3	4	6	55 <sup>d</sup>

<sup>a</sup>p<0.05, HC group and NC group, <sup>b</sup>p<0.05, NE group and NC group, <sup>c</sup>p<0.05, HE group and NE group, <sup>d</sup>p<0.05, HE group and HC group, <sup>e</sup>p>0.05, HC group and NE group. NC: Normoxic control, HC: Hypoxic control, NE: Normoxic experimental, HE: Hypoxic experimental; +: Weakly positive; ++: Generally positive; +++: Strongly positive

Table 4: Content of cytokines in Gingival Crevicular Fluid (GCF)

Group	TNF- $\alpha$ ( $\mu\text{g L}^{-1}$ )	PGE <sub>2</sub> ( $\mu\text{g L}^{-1}$ )	IL-8 ( $\mu\text{g L}^{-1}$ )
NC	0.1564±0.0372	0.8325±0.0294	3.9432±0.9095
HC	0.0816±0.0213 <sup>b</sup>	1.0157±0.0641 <sup>b</sup>	1.9698±0.3534 <sup>b</sup>
NE	1.0467±0.8105 <sup>a</sup>	1.8395±0.6514 <sup>a</sup>	0.8349±0.1516 <sup>a</sup>
HE	1.9698±0.3534	2.4716±1.1463	0.2150±0.0641

<sup>a</sup>p<0.05, compared with NC group, <sup>b</sup>p<0.01, compared with HE group. NC: Normoxic control, HC: Hypoxic control, NE: Normoxic experimental, HE: Hypoxic experimental

TNF- $\alpha$  was secreted largely from macrophages which were activated by hypoxemia, under hypoxic conditions. The cytokine concentrations in GCF of SD rats were determined based on the standard curves of OD values.

Table 4 shows that TNF- $\alpha$  and PGE<sub>2</sub> levels in the HE group were higher than those in the other groups. By contrast, the IL-8 level was lower in the HE group than in the other groups. A previous study (Haddad and Harb, 2005) also revealed a similar result, in which TNF- $\alpha$  and PGE<sub>2</sub> in GCF had a significantly positive correlation with the clinical periodontal parameters (BI, PLI, PD and AL), whereas IL-8 had a negative correlation.

Obstructive Sleep Apnea (OSA) which is characterized by a typical breathing pattern of intermittent hypoxia, results in alternating cycles of hypoxia and re-oxygenation during circulation. Previous studies reported that intermittent hypoxia, a unique form of hypoxia that occurs in OSA, induces the activation of transcription factors, such as nuclear factor kappa B (NF- $\kappa$ B). The serum levels of TNF- $\alpha$ , IL-8 and IL-6 consecutively increased from the onset to the sixth week in all hypoxic experimental groups in rat models (Li *et al.*, 2011).

In the present study, animal models for periodontitis were established in a simulated high-altitude environment. The GCF of SD rats from different groups was collected and the concentration of three kinds of cytokines (TNF- $\alpha$ , PGE<sub>2</sub> and IL-8) was detected. The results indicated that TNF- $\alpha$  and PGE<sub>2</sub> levels in GCF of the HE group were significantly higher than those in the NE group (p<0.01). These levels were positively correlated with AL, PLI and BI. By contrast, IL-8 level was lower in the NE group than in the control groups (p<0.01) and exhibited a negative correlation with the clinical periodontal parameters (p<0.01). These results indicated that higher altitudes may cause an increase in TNF- $\alpha$  and PGE<sub>2</sub> levels, as well as a decrease in IL-8 level. The concentrations of the inflammatory factors in GCF were different and thus correspond to the extent of periodontal destruction. The analysis of the relationship between the periodontal parameters (AL, PLI and BI) and the concentration of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 indicated that TNF- $\alpha$  concentration increases with the depth of the probe. This effect is caused by the subgingival anaerobes and

endotoxins which increase with the depth of periodontal pockets, thereby resulting in increased expression of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8. More inflammatory factors aggravate the damage and deepen the periodontal pockets. The overall process then becomes a vicious cycle. BI is a sensitive indicator for gingivitis. TNF- $\alpha$  and PGE<sub>2</sub> concentrations increase with the severity of gingivitis because BI is related to the number of mononuclear macrophages and lymphocytes. A previous study revealed that the lipopolysaccharide of *Porphyromonas gingivalis* can activate the proliferation of mononuclear macrophages and production of TNF- $\alpha$  (Roberts *et al.*, 1997). Numerous studies have also demonstrated that TNF- $\alpha$ , as a kind of Th1-type cytokines, participates in periodontal pathogenic process and facilitates osteoclast generation (Garlet *et al.*, 2005). In the present study, the TNF- $\alpha$  concentration under hypoxic conditions significantly increased in periodontal tissues because macrophages become activated in hypoxia-ischemia and excrete TNF- $\alpha$ . Osteoclasts are formed, thereby resulting in bone resorption.

Our results showed that the density of IL-8 in the experimental groups was lower than that in the control groups but other studies have reported different results (O'Brien-Simpson *et al.*, 2009). IL-8 can improve the immune defense of hosts against Gram-negative bacteria and prevent periodontal infection. However, neutrophils and phagocytic cells cannot be sufficiently induced and attracted if the density of IL-8 is extremely low. Thus, host defense against periodontal pathogens becomes weakened. Although neutrophils and phagocytic cells have reached the lesion sites, their functional activities decrease because of the insufficient IL-8 density (Mathur *et al.*, 1996). IL-8 is relatively unique because it may be produced early in the inflammatory response but persists for a prolonged period, for days or even weeks, in contrast to most inflammatory cytokines which are typically produced and cleared during *in vivo* situations within a few hours (Andia *et al.*, 2011).

Optional regulation and intervention on TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 can control periodontal inflammation and alveolar bone resorption under hypoxic conditions. Thus, the onset of periodontitis can also be controlled. This discovery provides new information to the study and the treatment of plateau periodontitis. However, further investigation should be conducted to determine the relationship between TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in hypoxic environments or whether their combined effects would lead to bone resorption and periodontitis.

Although the precise mechanisms remain unknown, several potential explanations account for the association between poor periodontal health status and plateau environment.

## CONCLUSION

In this study, we successfully established a rat periodontitis model in a simulated high-altitude hypoxic environment. In the hypoxic environment, TNF- $\alpha$  and PGE<sub>2</sub> levels increased, whereas IL-8 level decreased, indicating that the hypoxic environment may influence the expression and the secretion of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 in periodontal tissues. We found significant correlations of TNF- $\alpha$ , PGE<sub>2</sub> and IL-8 with clinical periodontal parameters which include BI, PLI, AL and PD, indicating that the difference in the expression and the secretion of these specific cytokines may modify the microenvironment of periodontal tissues. The results also indicate that hypoxia and high-altitude environment can accelerate and aggravate the pathophysiological process of periodontitis.

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