

## Original Article

# Expression of hBDs in gingival tissue in patients with cleft alveolus

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**Abstract:** To evaluate the relationship between expression level of hBDs (human beta defensin) and clinical indexes in gingival tissue in patients with cleft alveolar who also suffering from periodontitis, 35 patients with cleft alveolar (age range from 12-19 years) and 31 healthy subjects (age range from 13-18 years) were examined with clinical periodontal parameters. RT-PCR was performed to evaluate the expression level of hBDs in gingival tissue from both groups. Immunohistochemistry was also conducted to detect the hBDs expression level in gingival tissues. The clinical indexes of patients were collected again 3 months after surgery. There was a significant difference between healthy subjects and patients before surgery. In group of patients with cleft alveolar, the clinical indexes were improved after surgery. The expression level of hBD-1, 2 and 3 were also changed to the level similar with healthy control. Our results indicated that periodontitis could be very serious in patients with cleft alveolar before surgery without special treatment. Gingival condition could be improved but there still a difference between patients after surgery and health control. The gingival health should be maintained as a part of sequence treatment of cleft lip and palate.

**Keywords:** Periodontitis, cleft alveolar, cleft lip and palate

## Introduction

Cleft lip and palate is a kind of congenital craniofacial deformity with very high frequency all around the world. Patients suffer not only from the appearance deformity, but also from physiological dysfunction. The management of patients with cleft needs comprehensive treatment with highly specialized and cooperated teamwork. The comprehensive treatment began from birth and will last to adult or longer including the basic maintain of periodontics' condition. Only in this way patients could achieve the best result.

After primary cheiloplasty and palatoplasty, cleft alveolar will be repaired during the period of mixed dentition. A discontinued arch may result in occlusal dysfunction and difficulty of cleaning. Besides these, patients always suffer from gingival bleeding and lose of attachment level [1, 2]. The periodontitis accompanied by

cleft alveolar, is usually caused by bad oral hygiene statue because of abnormal oral structure [3, 4]. Many researchers have reported that patients with cleft suffered from periodontitis during their adolescence [5, 6]. In China, because of the lower medical development and lack of relative research, the situation could be much more serious.

In patients with cleft alveolar, due to the irregular occlusion relationship, it is difficult to clean the teeth and tooth-supporting tissues [7, 8]. Bacteria accumulated and destructed the gingival barrier and then caused gingivitis and periodontitis [9]. Other factors may worsen this condition, including orthodontic appliances, removable and irremovable prostheses [10]. Orthodontic appliance and prostheses can restore part of function of occlusion but also have a negative influence on oral hygiene. Especially the teeth adjacent to the cleft, usually shows a significant loss of attachment and

periodontal destruction [11, 12]. Though few researches reported that there was no difference between patients with cleft and subjects without cleft [8], others reported that patients with cleft were more susceptible to periodontitis [1, 13, 14].

Most of researches have reported the clinical examination result without detection of periopathogens in patients with cleft alveolar. As we already known, the change the expression of periopathogens is an indispensable part of periodontitis. During the progress of periodontitis, human  $\beta$ -defensin (hBDs) could be the first line of defense and their expression will change with the development of periodontitis [15, 16]. The hBDs could be detected in epithelia, salivary glands, saliva and gingival crevicular fluid. When the gingival barrier was destructed by a certain kinds of bacteria and pathogens, the periodontitis occurs and the expression level of hBDs changed accordingly [17, 18]. This is a characteristic change of periodontitis. If this condition has not been reversed, the tooth-supporting tissues would be destroyed and result in serious and irreversible tooth-supporting tissue degeneration and missing teeth. To evaluate the condition of periodontitis, it is necessary to evaluate the expression level of human  $\beta$ -defensins (hBDs) [17].

The aims of present study were 1) to compare the overall periodontal condition in patients with cleft alveolar before and after bone grafting surgery and 2) to compare the periodontal condition between patients and healthy control by using clinical indexes and histological characteristics.

### Subjects and methods

#### *Subjects*

35 patients (age range from 12-19 years) with untreated unilateral cleft alveolar were selected from the department of oral and maxillofacial surgery, College of Stomatology, Harbin Medical University, China. All of patients have already finished primary surgery of cleft lip and palate. Before surgery of cleft alveolar, they had never accepted any other kinds of surgical or non-surgical treatment except for primary cheiloplasty and palateplasty. There were no other systemic diseases except for cleft. All of patients had no antibiotic therapy and immuno-

suppressive therapy in recent one year. 41 health subjects (age range from 13-18 years) were selected as control group. This group was recruited from clinic department of oral and maxillofacial when they came for extraction of impacted molar. All of subjects followed routine oral health care. Before tooth extraction, they didn't accept any treatment related to oral disease.

Written and oral informed consents were obtained from all subjects and their parents for this study. The study was approved by the Ethics Committee of the Faculty of Hospital. There was no financial support for this study could have influenced its outcomes.

#### *Parameters record*

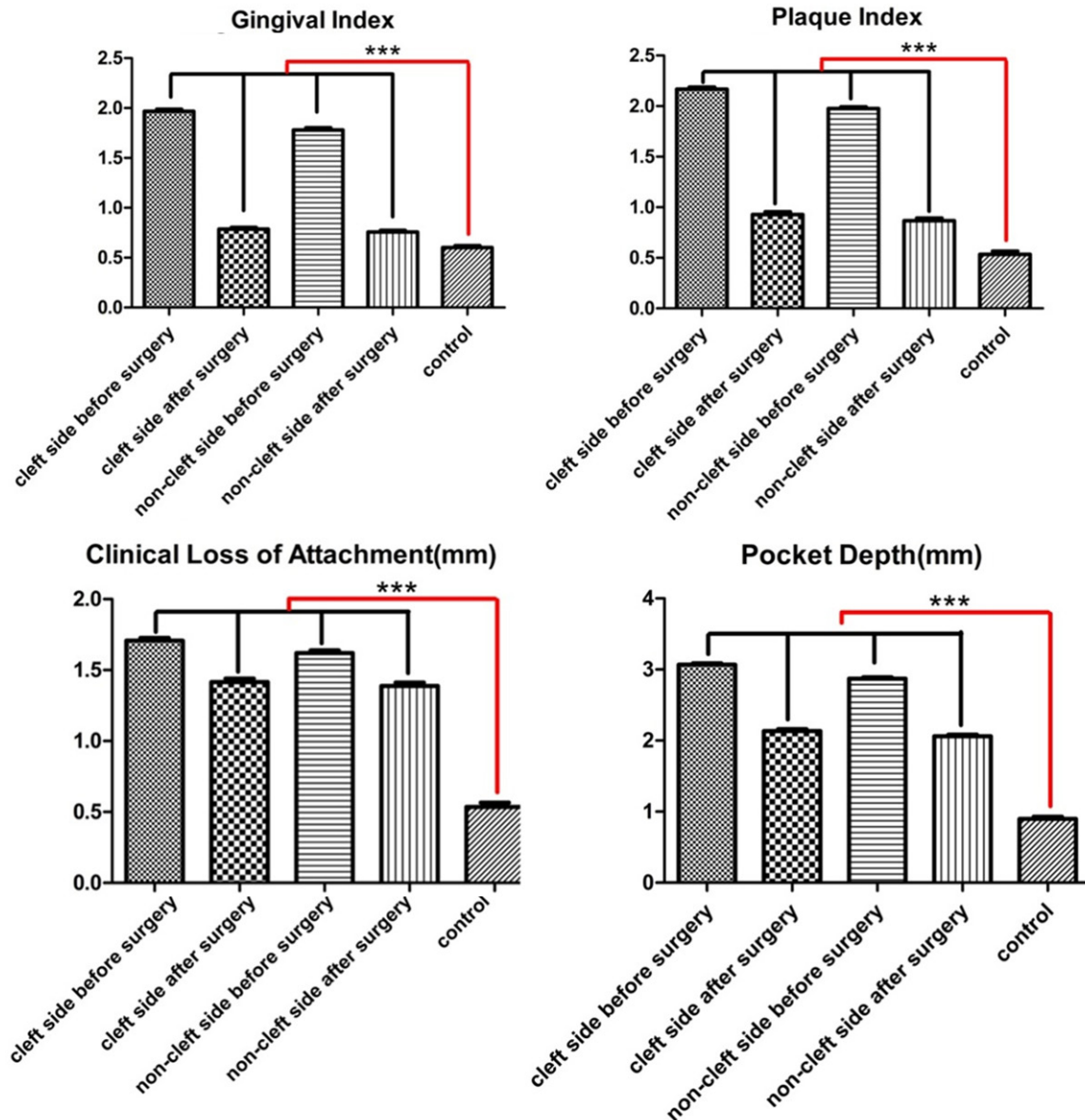
A serious of oral indexes was used to evaluate the oral hygiene. For each subjects in this study, the following parameters were recorded: plaque index (PLI), gingival index (GI), pocket depth (PD), clinical loss of attachment (CLA). All the indexes were recorded at the canine adjacent immediately to the cleft and the canine at the other side.

For patients with cleft alveolar, the recording began 1 week before the surgery and 3 months after surgery. For subjects of control group, the parameters were recorded when they were recruited in this study and repeated 3 months later to match the time point of patients with cleft alveolar. The average result of two measurements was taken as final parameters.

#### *Collection of samples*

For each subject in patients group, gingival samples were harvested during the surgery of bone grafting in patients group. Before suture of incision, the gingival flap should be trimmed and the sample could be harvested for present study. In healthy control group, gingival samples were harvested when the impacted molar was extracted. Like experiment group, the tissue sample were harvested when the wound of extraction were trimmed.

For IHC, gingival tissues were fixed by formalin as regular protocol. All tissues were embedded by paraffin for section preparation as regular protocol. The IHC was performed in department of pathology to detect the expression of hBDs.



**Figure 1.** Clinical parameters in patients with cleft alveolar and healthy control. Red line indicates that there is significant difference between control group and patients groups. In all four indexes, significant difference could be found between control group and patients groups no matter before or after surgery, on cleft side or non-cleft side. Gingival index and Plaque index: the difference was obviously before surgery when compared to control group. 3 months after surgery, though there still significant difference, the data became very close to the normal control. Clinical loss of attachment: it was difficult to restore the attachment even after the surgery. Pocket depth: the depth of pocket reduced but there was still significant difference when compared to the normal control.

#### *Surgery for cleft alveolus*

The surgery procedure for cleft alveolar followed classic procedure in our hospital. In briefly, the granular bone marrow was harvested from anterosuperior iliac spine by a group of surgeon. At the same time, another group of surgeon was preparing the pocket for bone

grafting at the area of cleft alveolus. Then the bone marrow was implanted into the pocket and watertight suture were applied to protect the surgical area. A set of orthodontic wire and bracket were applied to make sure that the bone segments be kept in a stable position. After two or three day's observation after surgery, patients were discharged and were asked

## Periodontitis in cleft patients

**Table 1.** The clinical parameters from patients group before and after surgery and from control group

	gingival index	plaque index	pocket depth (mm)	clinical loss of attachment (mm)
cleft side before surgery	1.968±0.121	2.17±0.12	3.07±0.12	1.71±0.12
cleft side after surgery	0.7916±0.101	0.93±0.16	2.13±0.17	1.42±0.16
non-cleft side before surgery	1.78±0.135	1.98±0.11	2.87±0.12	1.62±0.12
non-cleft side after surgery	0.76±0.097	0.86±0.15	2.06±0.14	1.39±0.14
control	0.61±0.1	0.53±0.17	0.9±0.17	0.52±0.17

**Table 2.** The comparison of clinical parameters between groups

	cleft side before surgery	cleft side after surgery	non-cleft side before surgery	non-cleft side after surgery	control
cleft side before surgery		GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA
cleft side after surgery	GI, PLI, PD, CLA		GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA
non-cleft side before surgery	GI, PLI, PD, CLA	GI, PLI, PD, CLA		GI, PLI, PD, CLA	GI, PLI, PD, CLA
non-cleft side after surgery	GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA		GI, PLI, PD, CLA
control	GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA	GI, PLI, PD, CLA	

The word in red indicated there was significant difference, and the word in black indicated there was no significant difference. According to the table, except CLA, there were significant difference in GI, PLI and PD between both sides before surgery ( $P<0.05$ ). 3 months after surgery, there were no difference between both sides. When compared to normal control, no matter before and after surgery, there were significant difference even 3 months observation finished ( $P<0.05$ ).

to return 1 month later to remove the orthodontic tire and bracket. 3 months after surgery, patients were required to return and the periodontal parameters were collected again.

### Immunohistochemical assay

The tissues from healthy control group and patients group with cleft alveolar were fixed and the 5  $\mu$ m thick sections were prepared. Immunohistochemistry was conducted as standard protocol. In present study, the hBD-1, hBD-2 and hBD-3 were examined by using rabbit antiserum. Anti-mouse hBD-1, hBD-2 and hBD-3 monoclonal antibody (1:100 dilution; Abcam, Cambridge, UK) was used for immunohistochemical detection of gingival tissues. Specificity was evaluated by substituting PBS for each primary antibody. All the sections were analyzed under light microscopy.

### Statistical analysis

All data were collected and analyzed with SPSS (SPSS 10). The mean values of clinical parameters (GI, PLI, PD, and CLA) were analyzed with a paired Student's *t*-test. A *P*-value less than 0.05 were considered significant.

### Results

A total 35 patients and 31 healthy subjects were included in the present study. All of param-

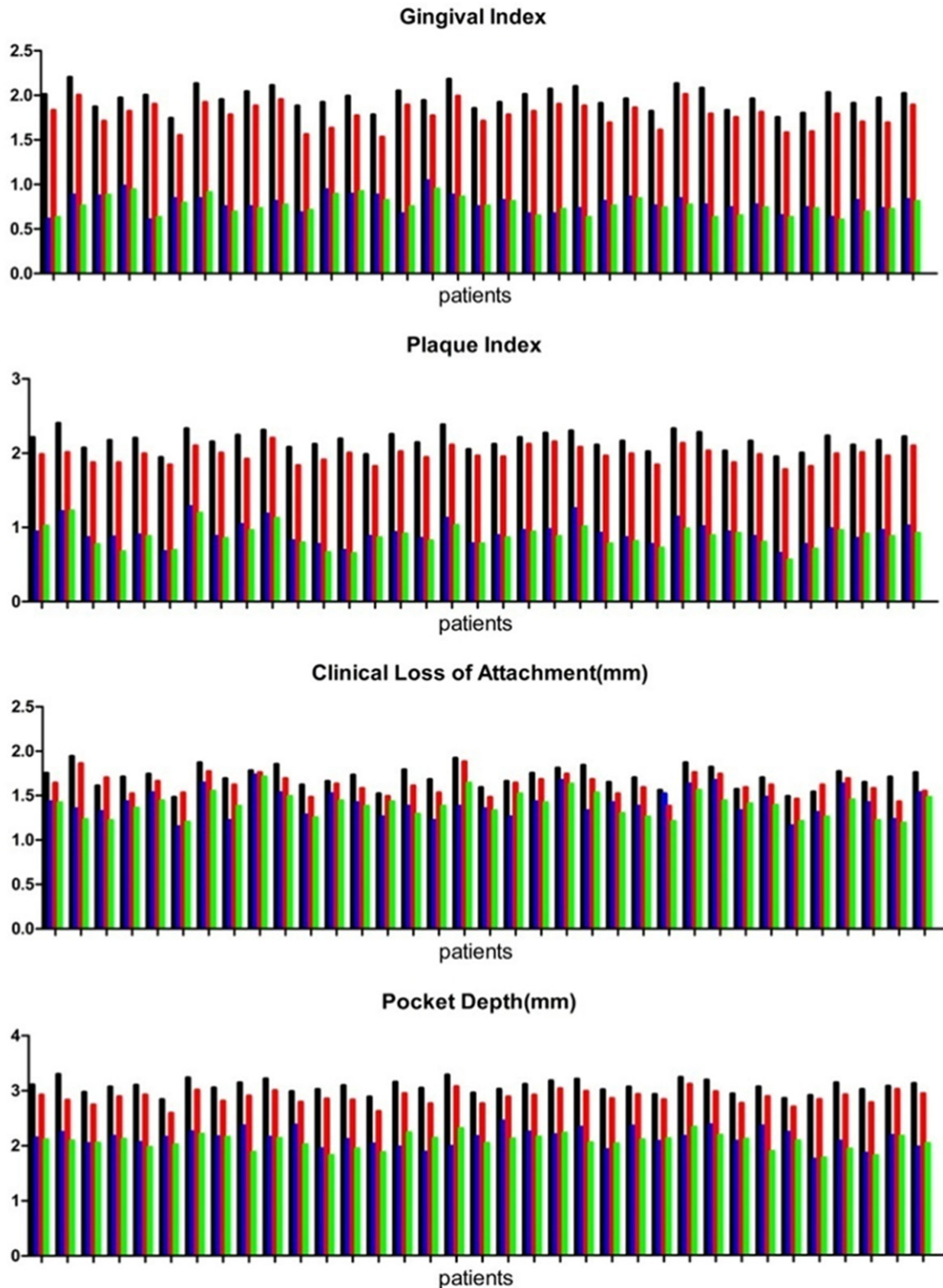
eters in patients group were examined and recorded 1 week before surgery and 3 months after surgery. In healthy group, the parameters were examined and recorded at the proper time to match the time point in patients group.

### Abnormal anatomic resulted in bad oral hygiene and periodontitis

In patients group, the clinical parameters indicated a serious periodontitis. As we can see in **Figure 1**, the GI, PLI, PD and CLA indicated a serious periodontitis in patients with cleft alveolar, no matter on the cleft side or non-cleft side. There was no significant difference between both sides before surgery except CLA (**Table 2**). But the significant difference could be found between patients group and healthy control. The mean gingival index was 1.968±0.121 and 1.78±0.135 before surgery on both sides in patients with cleft, but was 0.61±0.1 in control group (**Table 1**). Same condition could be found in plaque index, pocket depth and clinical loss of attachment.

Result of IHC indicates a histological change in gingival tissues. The expression level of hBD-1 and hBD-3 decreased in patients with cleft alveolar before surgery, compared to the normal control. On the contrary, the expression level of hBD-2 kept in a low level before surgery.

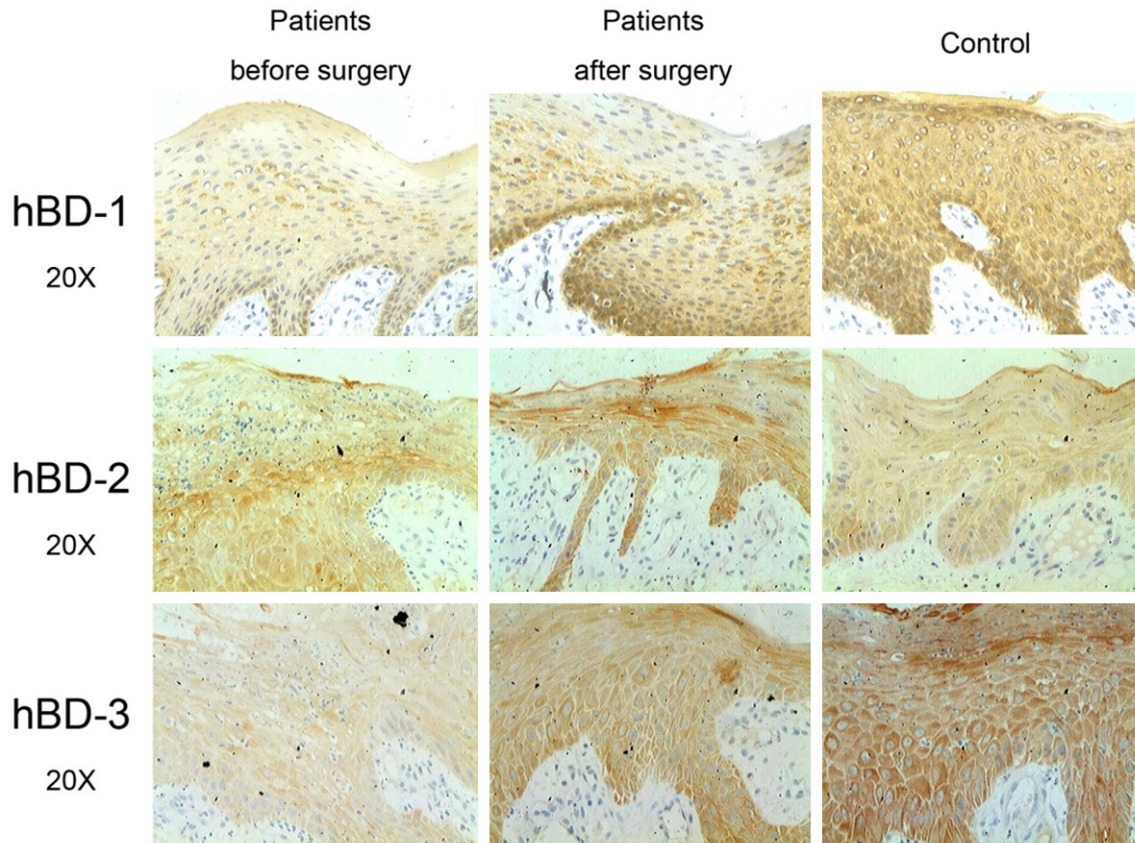
## Periodontitis in cleft patients



**Figure 2.** Comparison between cleft side and non-cleft side in patients. Black column represents index before surgery on cleft side, red column represents index before surgery on non-cleft side. Blue column represents index after surgery in cleft side, green column represents index after surgery in non-cleft side. This kind of comparison was used to indicate though there was significant difference between both sides in patients with cleft alveolar before surgery, the data were very close. 3 months after surgery, the four indexes improved simultaneously to a relative lower level.



## Periodontitis in cleft patients



**Figure 3.** Immunohistochemical assay indicated expression of hBDs in gingival tissues. In patients before surgery, the expression level of hBD-1 and hBD-3 decreased because of periodontitis but hBD-2 increased its expression level. 3 months after surgery, the expression level return to a level close to the normal control, indicated that the periodontitis was improved. Besides the expression of hBDs, the change of number of inflammatory cell could be used to indicate the improvement of periodontitis in gingival tissues.

This feature of expression indicates the periodontitis around the teeth supporting tissues.

*Surgeries of alveolar bone graft repair the cleft alveolar together with the improvement of oral hygiene.*

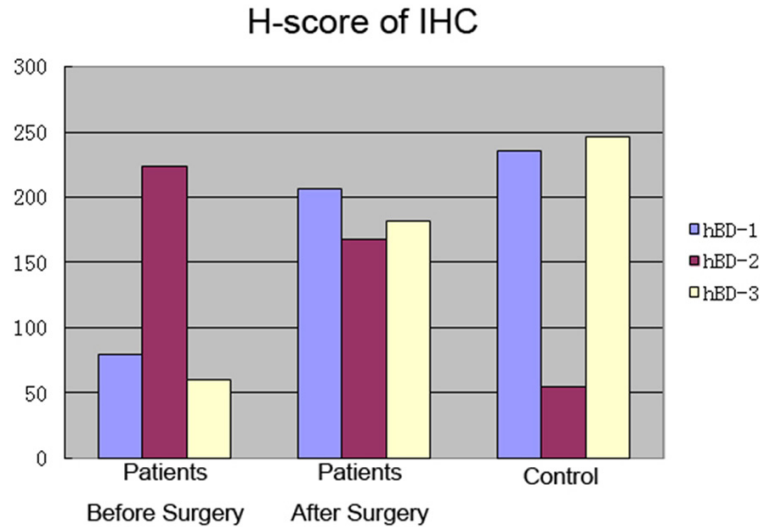
3 months after surgery, the conditions were improved in patients group (**Table 1**). All of the four indexes were decreased and closed to normal level, as we can see in **Figures 1, 2**. Compared to the indexes before surgery, there was significant difference between both sides. There were no significant difference could be found between both sides in patients. And when compared with normal control, there was still significant difference could be found (**Figures 1, 2**).

The IHC result also indicated that the expression level of hBD-1 and hBD-3 were recovered

after surgery though expression level of hBD-2 decreased closed to normal level, compared to the normal group.

*Compare to the control group, patient group still have obvious difference*

When compare to the normal control group, all of data from patients group showed significant difference. Before the surgery, there were worse oral hygiene conditions in patients with cleft alveolar. All of clinical indexes showed significant difference when compared to the control group. 3 months after surgery, the comparison between control and patients still showed a significant difference but the gap has already been narrowed, as shown in **Table 1**. The GI, PLI, PD and CLA were  $1.968 \pm 0.121$ ,  $2.17 \pm 0.12$ ,  $3.07 \pm 0.12$  and  $1.71 \pm 0.12$  on cleft side in patients before surgery, and these data changed to  $0.7916 \pm 0.101$ ,  $0.93 \pm 0.16$ ,



**Figure 4.** H-score of IHC staining.

2.13±0.17 and 1.42±0.16 after surgery. Though there still significant difference, but the number of indexes already became very close to normal condition.

The result of IHC demonstrated a restore of expression level of hBDs: in **Figures 3 and 4**, the hBD-1 and hBD-3 showed a relative high level in gingival tissues and hBD-2 showed a relatively lower level after surgery.

### Discussion

Patients with cleft alveolar have an abnormal anatomic structure of oral cavity [19]. The discontinued arch makes it difficult to clean the teeth and oral cavity as health people do [20]. Before the primary cheiloplasty, the end of segments of arch always become distorted or exposed to outside of oral cavity directly. Because the cleft make oral cavity an open space, patients suffer from gingival atrophy, gingivitis or even periodontitis in the early age of patients. The gingivitis or periodontitis could be found not only at the teeth adjacent to cleft on the cleft side immediately, but also the teeth far from the cleft on the non-cleft side [14]. As **Figure 1** indicated, there was no significant difference between data from both sides of patients with cleft alveolar. Along with the clinical finding, expression level of human beta defensins (hBDs) also shows a significant change in gingival tissues. At normal condition, hBD-1 and hBD-3 keep in a high level of expres-

sion compare to the low level of hBD-2. In periodontitis, the expression level reversed. So the hBDs could be used as a marker in pathological diagnosis of periodontitis.

The periodontitis in patients with cleft alveolar is a widespread disease. Cleft alveolar, together with cleft lip and palate, is one of the most common congenital deformities all around the world. Besides the deformity of lip and nose, the over-opening oral environment will cause a serious chronic disease such as periodontitis, which will result in atrophy of tooth support tissue and early tooth loss [3, 4].

In 2003, Salvi GE, et al, reported that the cleft lip, alveolar and palate (CLAP) and the cleft lip/palate (CL/CP) subjects are at high risk for periodontitis. Their clinical finding indicated that high plaque index and gingival inflammation scores could be found at cleft sites, and then result in more destruction of periodontal tissue than in control sites over a 14-year period [1]. The bad oral hygiene should be improved in patients with cleft [8].

In present study, we recorded the clinical parameters before and after the surgery of cleft alveolar repair. **Table 1** shows all of the clinical parameters in patients with cleft alveolar before and after the surgery.

The marked changes of PLI, GI, PD and CLA could be found between different time points and different groups. According to the data, before the surgery, the periodontal condition was very serious in patients. Distorted anatomical structure had a negative influence on maintain of oral hygiene. Besides the clinical parameters, histological change also could be found and was consistent to the clinical findings (**Figures 3 and 4**).

After reconstruction of upper arch, the bone segments reunited together and the function of occlusion could be restored [21, 22]. Significant changes in the patient group from beginning to 3 months after surgery were found for all mea-

tures examined (**Figure 1**). At the beginning of present study, there was significant difference between patients with cleft alveolar and health control subjects. The clinical parameters showed a big gap in data and significant difference between groups (**Table 1**). The expression level of hBDs also proved that the periodontitis not only worsen the clinical parameters, but also reversed the expression style of hBDs. And 3 month after surgery of cleft alveolar repair, this significant difference disappeared, indicates that the oral health statue has been improved after anatomy structure was restored [23]. This change could be found when compared the difference between both sides in patients also. **Table 2** indicated that after surgery, the all of four clinical parameters became very close in both sides. This result demonstrated that the surgery promoted the overall level of oral health. The expression style of hBDs restored after the surgery, too. Our finding indicated that reconstruction of cleft alveolar improved not only clinical indexes, but also changed the histological feature of periodontitis. Oral mucosal barrier could be restored at the same time.

In patients with cleft alveolar, histological changes in periodontitis was same with chronic periodontitis and was consistent with clinical features. In present study, all four clinical parameters were typical feature of chronic periodontitis. Gingival index and plaque index were higher than healthy control. Then expression level of hBDs reversed when compared with normal control. The hBDs were barriers of gingival epithelia against bacteria. Several studies have shown that hBDs play major roles in the chemotactic immune and inflammatory responses of immature dendritic cells, memory T-cells, and monocytes. These expression styles were similar with present study. In present study, the expression frequencies of the hBD-1 and hBD-3 increased after surgery of cleft alveolar. This result indicates that hBD-1 and hBD-3 are required for antimicrobial in gingival tissues but their expression were reduced by periodontitis. 3 months after surgery and periodontal therapy, the level of expression of these two defensins increased. This kind of change is also similar with chronic periodontitis without cleft alveolar. Besides this, the inflammatory cell infiltration could be found in tissues from patients with periodontitis. This condition was improved after surgery (**Figures 3 and 4**).

There is still no report about whether pathological feature in patients with cleft alveolar is similar or different with chronic periodontitis. Present study tried to evaluate the clinical and histological feature in patients with cleft. Compared with health subjects in control group, the findings of this study indicated that the periodontitis were similar with chronic periodontitis in patients without cleft alveolar, but it was difficult to reverse this condition only by surgery. We observed for 3 months after surgery, there was still significant difference between patients groups and health group (**Figures 1 and 2**). This indicated that, to eradicate the periodontitis in patients with cleft alveolar, the surgery of cleft alveolar repair was not enough [24].

In conclusion, the clinical features and pathological change in gingival tissues were similar with chronic periodontitis in patients with cleft alveolar. After the repair of cleft alveolar, periodontal tissue condition could be improved, leaving narrowed gap when compared with normal subjects. Our results indicated that early comprehensive treatment is necessary for patients with cleft alveolar.

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### Disclosure of conflict of interest

None.

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