ระดับคอนดรอยตินซัลเฟต (WF6 เอพิโทป) ที่เพิ่มขึ้นรอบฟันกรามบนและวัสดุฝังเกลียวขนาดเล็กระหว่างการดันฟันกรามบนเข้าเบ้าฟันทางทันตกรรมจัดฟัน

Raised Chondroitin Sulphate (WF6 epitope) Levels around Maxillary Molars and Miniscrew Implants during Orthodontic Molar Intrusion

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบระดับคอนดรอยตินซัลเฟต (WF6 เอพิโทป) ในน้ำเหลืองเหงือกรอบฟันกรามบนและวัสดุฝังเกลียวขนาดเล็กระหว่างการดันฟันกรามเข้าเบ้าฟันทางทันตกรรมจัดฟัน ผู้ป่วยที่มีโครงสร้างศีรษะแบบเปิดสิบรายซึ่งต้องรักษาด้วยการดันฟันกรามเข้าสู่เบ้าฟันเข้าร่วมในการศึกษานี้ วัสดุฝังเกลียวขนาดเล็กหนึ่งตัวถูกฝังที่กลางเพดานปากและใช้สปริงเซนทัลลอยชนิดปิด (100 กรัม) สองตัวเพื่อดันฟันกรามเข้าเบ้าฟัน ทำการเก็บน้ำเหลืองเหงือกรอบฟันกรามทดลอง ฟันกรามควบคุม

Abstract

This study aimed to monitor chondroitin sulphate (CS; WF6 epitope) levels in crevicular fluid around maxillary molars and miniscrew implants during orthodontic molar intrusion.

One miniscrew implant was placed in the midpalatal area of each of ten patients with open skeletal configurations, who required orthodontic molar intrusion, and two Sentalloy® closed-coil springs (100 g) were used for molar intrusion. Gingival crevicular fluid (GCF) around experi-

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และวัสดุฝังเกลียวขนาดเล็กก่อนและระหว่างให้แรงเคลื่อนฟัน ตัวอย่างทั้งหมดถูกนำไปวิเคราะห์หาระดับคอนดรอยตินซัลเฟต (WF6 เอพิโทป) โดยวิธีคอมเพททิทีปอีไลซ่าร่วมกับโมโนโคลนอลแอนติบอดี้ WF6 ผลการศึกษาพบว่า ค่ามัธยฐานของคอนดรอยตินซัลเฟต (WF6 เอพิโทป) รอบฟันกรามทดลองในช่วงให้แรง 12 สัปดาห์ (2.099 นาโนกรัม/ไมโครกรัมโปรตีน) และค่าในช่วงให้แรงทุกสองสัปดาห์ (1.952, 1.854, 2.604, 2.414, 1.844, 1.44 นาโนกรัม/ไมโครกรัมโปรตีนตามลำดับ) มากกว่าค่าในช่วงก่อนให้แรง (0.832 นาโนกรัม/ไมโครกรัมโปรตีน) อย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ในขณะที่ค่ามัธยฐานของคอนดรอยตินซัลเฟต (WF6 เอพิโทป) รอบฟันควบคุมและวัสดุฝังเกลียวขนาดเล็กไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างสองช่วง ผลการศึกษานี้อาจเน้นย้ำให้เห็นถึงบทบาทการเป็นตัวชี้วัดทางชีวภาพของคอนดรอยตินซัลเฟต (WF6 เอพิโทป) สำหรับการละลายของกระดูกรอบฟันที่ถูกเคลื่อนทางทันตกรรมจัดฟันรวมถึงรอบวัสดุฝังเกลียวขนาดเล็ก

คำสำคัญ: คอนดรอยตินซัลเฟต (WF6 เอพิโทป) การดันฟันกรามเข้าสู่เบ้าฟัน น้ำเหลืองเหงือก ตัวชี้วัดทางชีวภาพ

mental and control molars, and peri-miniscrew implant crevicular fluid (PMICF) were collected before and during load application. Competitive ELISA with monoclonal antibody WF6 and colorimetric protein assay were used to detect CS (WF6 epitope), and total protein concentration, respectively.

The results showed that the median CS (WF6 epitope) levels around experimental molars during the loaded period (12 weeks) (2.099 ng/μg of total protein) and those during each two-week interval of the loaded period (1.952, 1.854, 2.604, 2.414, 1.844, 1.44 ng/ μg of total protein respectively) were significantly greater than those during the unloaded period (2 weeks) (0.832 ng/ μg of total protein) ($P<0.05$), whereas the median CS (WF6 epitope) levels around control molars and around miniscrew implants, during the unloaded and loaded periods, were not significantly different. The results of this study may emphasize the role of CS (WF6 epitope) level as a biomarker for alveolar bone resorption around orthodontically moved teeth and also around miniscrew implants.

Keywords: Chondroitin sulphate (WF6 epitope), molar intrusion, gingival crevicular fluid, biomarker

Introduction

Molar intrusion is an option for treating open skeletal configuration cases. During molar intrusion, anchorage control is very important. Recently, miniscrew implants have been widely used in many orthodontic treatments, including molar intrusion, to provide absolute anchorage in order to minimize the need for patient compliance. Many investigators have reported successful results of miniscrew implant anchorage for molar intrusion.[1-5] However, those investigations assessed molar intrusion by using only clinical and radiographic parameters.

Orthodontic force causes metabolic changes in periodontal tissue. This force results in alterations of biochemical components of gingival crevicular

fluid (GCF), which can be assessed by monitoring inflammatory mediators, enzymes and tissue breakdown products in GCF.[6] Chondroitin sulphate (CS) is the main component of glycosaminoglycans in alveolar bone and can serve as a marker for active alveolar bone and periodontal ligament turnover.[7] CS levels have been used to investigate alveolar bone remodeling as a result of periodontal disease and orthodontic tooth movement.[7-8] Last et al.[9] showed a significant rise in chondroitin sulphate levels in the gingival crevicular fluid of teeth undergoing orthodontic tooth movement. Jaito et al.[10] reported that the detectable CS levels were associated with the applied orthodontic forces. For miniscrew implants, Intachai et al.[11] reported that the CS (WF6 epitope) could be detected in peri-miniscrew implant crevicular fluid, and CS (WF6 epitope) levels of one failed miniscrew implant were remarkably elevated 14 days prior to miniscrew implant failure. For detecting CS by the ELISA method, either monoclonal antibody 3B3 or WF6 have been used.[10,12] Monoclonal antibody WF6 (a product of the Thailand Excellence Center for Tissue Engineering, Faculty of Medicine, Chiang Mai University), developed against embryonic shark cartilage proteoglycans, has been applied as a biomarker for recognizing an epitope in CS chains. Using ELISA with monoclonal antibody WF6, trace amounts of glycosaminoglycans presenting in GCF can be quantified.[8] Previous studies have reported that CS (WF6 epitope) can be detected in GCF around orthodontically moved teeth and in peri-miniscrew implant crevicular fluid (PMICF) around miniscrew implants.[10-11] Accordingly, the aim of the present study was to biochemically assess tooth movement and miniscrew implant stability during orthodontic molar intrusion by monitoring CS (WF 6 epitope) levels in GCF and PMICF.

Materials and Methods

The study was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University. Informed consent was obtained from all patients.

Subjects

A total of ten adult patients with open skeletal configuration and with anterior open bite, who required orthodontic molar intrusion, were included in the study. The patients met these following criteria: good general health and oral health with a healthy periodontium; no radiograhic evidence of bone loss; no gingival inflammation and a probing depth of 3 mm or less around all teeth; no antibiotic therapy during the previous 6 months; no anti-inflammatory drug administration in the month preceding the study; and no pregnancy (women).

Methods

A transpalatal arch with soldered hooks was inserted and one miniscrew implant (1.6 mm in diameter and 6.0-9.0 mm in length; Renew Biocare Corp., San Bruno, Cal., USA) was placed in the midpalatal area of each patient. During the unloaded period (2 weeks), the GCF around the right and left maxillary molars (as experimental molars) and around right mandibular first molars (as control molars) was collected on day 0, prior to intrusion. Then GCF and PMICF were collected on Days 1, 4, 7 and 14 after miniscrew implant placement. On Day 14, two Sentalloy® closed soil springs (100 g) (Tomy, Tokyo, Japan), connected from the miniscrew implant head to the soldered hooks, were used for molar intrusion (Figure 1). During the loaded period, the GCF and the PMICF were collected every week for 12 more weeks (Figure 2).

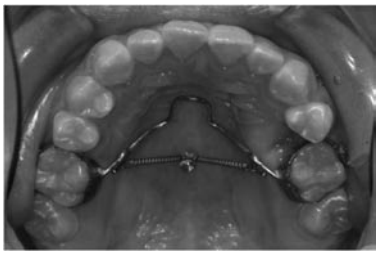

Figure 1 *Molar intrusion mechanics.*

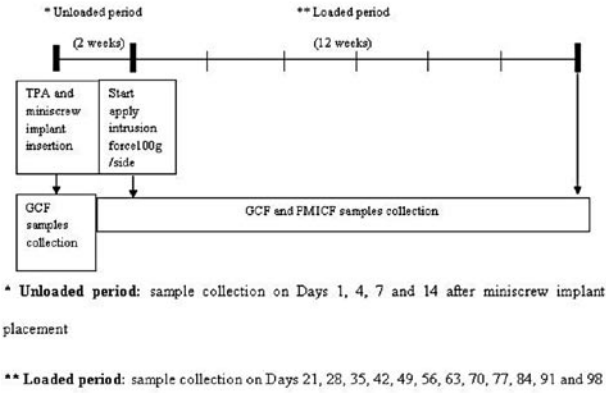

Figure 2 *Diagram of the experimental design.*

During GCF and PMICF collection, in both the unloaded and loaded periods, the experimental and control sites were isolated from saliva and gently air dried. The samples were collected by using 10.0x1.0 mm Whatman® No.1 (Whatman International Ltd, Maidstone, Kent, UK) filter paper strips inserted into the mesial gingival sulcus and peri-miniscrew implant sulcus. The last 2.0 mm of filter paper strip, containing either GCF or PMICF, was cut off and individually frozen at -80°C in a microcentrifuge for further analysis. Competitive ELISA with monoclonal antibody WF6 was used to detect CS (WF6 epitope), and colorimetric assay (Bio-Rad Protein Assay Kit II®, Bio-Rad, Hercules, Cal., USA) was used to detect total protein concentration. Miniscrew implant mobility assessment was performed after collecting PMICF sample at every visit by using cotton forceps.[13] Extremely light force was laterally applied to the miniscrew implant head. Mobility was assessed as either 'yes' (mobile) or 'no' (immobile). If there were any mobility, the miniscrew implant was categorized as mobile, and removed whereas miniscrew implants that were maintained in the bone until the end of the study period were considered to be successful.

The CS (WF6 epitope) levels in all samples were measured in nanogram per microgram (ng/μg) of total protein content. The differences in the CS (WF6 epitope) levels during the unloaded and the loaded periods were determined by the Wilcoxon signed-rank test. Results were considered statistically significant at $P<0.05$.

Results

Ten miniscrew implants were placed in midpalatal areas of six female and four male patients with mean age of 19.0±2.62 years (range from 15.6 to 24.3 years) (Table 1). No patient reported pain or discomfort at the time of sample collection. At placement and during the unloaded period, all miniscrew implants were clinically immobile. During the loaded period, one miniscrew implant was mobile and later removed on Day 18. The success rate of miniscrew implants was 90%. The positions of experimental molars before and after 12 weeks' applied intrusion force are shown in Figure 3.

Boxplot graphs of the median CS (WF6 epitope) levels per total protein in GCF around experimental molars, control right mandibular first molars, and PMICF around miniscrew implants during the unloaded and the loaded periods are shown in Figure 4.

Table 1 *Age (year) distribution by sex and number of the subjects (n) in each group in the present study.*

Gender	n	Minimum	Maximum	Mean	Standard Deviation
Female	6	17.5	24.3	20.2	2.35
Male	4	15.6	22.1	18.4	2.71
Total	10	15.6	24.3	19	2.62

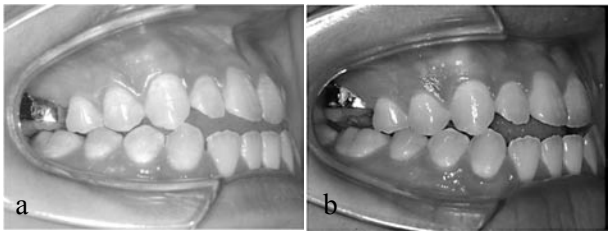

Figure 3 *Experimental molar before (a), and after (b), 12 weeks' applied intrusion force.*

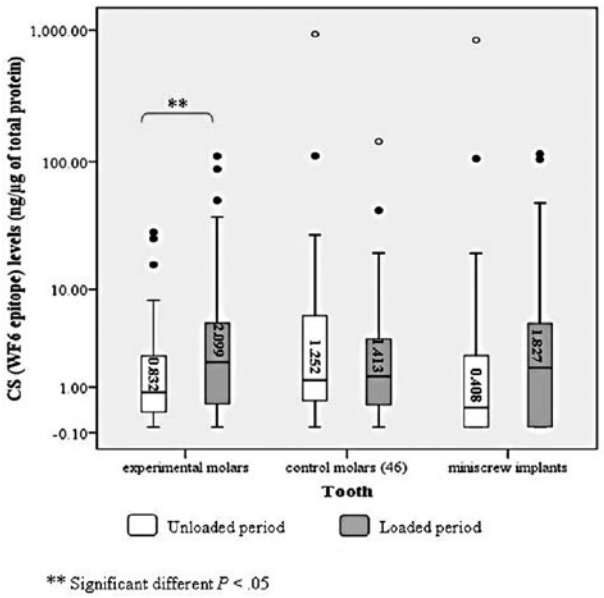

Figure 4 *Boxplot graphs of median CS (WF6 epitope) levels during the unloaded (2 weeks) and the loaded (12 weeks) periods around experimental molars, control right mandibular first molars and miniscrew implants.*

During the unloaded period, the CS (WF6 epitope) levels around experimental molars ranged from 0.0 to 28.6 ng/µg of total protein content and the median CS (WF6 epitope) level was 0.832 ng/µg (n = 90). The CS (WF6 epitope) levels around control right mandibular first molars ranged from 0.0 to 932.2 ng/µg of total protein content and the median CS (WF6 epitope) level was 1.252 ng/µg (n = 45). The CS (WF6 epitope) levels around miniscrew implants ranged from 0.0 to 836.0 ng/µg of total protein content and the median CS (WF6 epitope) level was 0.408 ng/µg (n = 36).

During the loaded period, the CS (WF6 epitope) levels around experimental molars ranged from 0.0 to 110.0 ng/mg of total protein content, and the median CS (WF6 epitope) level was 2.10 ng/µg (n = 212). The CS (WF6 epitope) levels around control mandibular right first molars ranged from 0.0 to 143.3 ng/µg of total protein content and the median CS (WF6 epitope) level was 1.413 ng/µg (n = 106). The CS (WF6 epitope) levels around minscrew implants ranged from 0.0 to 114.44 ng/µg of total protein content and the median CS (WF6 epitope) level was 1.827 ng/µg (n = 106).

The median CS (WF6 epitope) level around experimental molars during the loaded period (12 weeks) was significantly greater than that during the unloaded period (2 weeks) (P<.05). Around control molars and miniscrew implants, the medians of CS (WF6 epitope) levels during the loaded period were not significantly different from those during the unloaded period.

Boxplot graphs of median CS (WF6 epitope) levels per total protein in GCF and PMICF around experimental molars, control right mandibular first molars, and miniscrew implants during the unloaded period (2 weeks) and each two-week interval of the loaded period (12 weeks) are shown in Figures 5 to 7 respectively.

In Figure 5, median CS (WF6 epitope) levels around experimental molars during each two-week interval of the loaded period (12 weeks) were significantly greater than those during the unloaded period (2 weeks), whereas, there were no significant differences in the control molar and miniscrew implant groups (Figures 6 and 7).

Discussion

CS is the main component glycosaminoglycans in alveolar bone. The levels of CS in human GCF

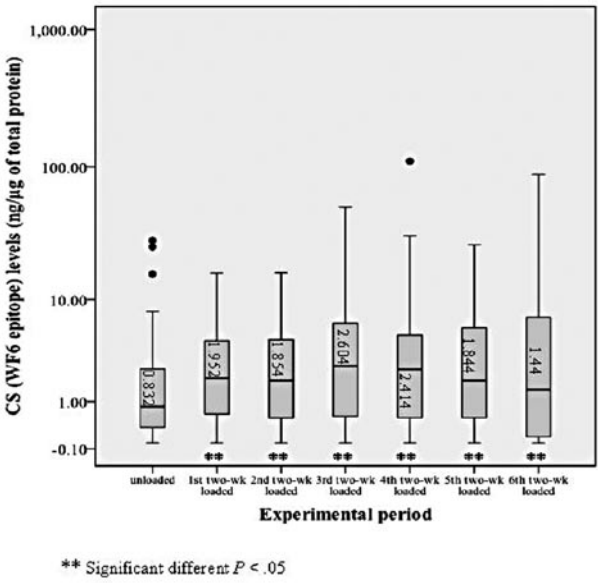

Figure 5 *Boxplot graphs of median CS (WF6 epitope) levels during the unloaded period (2 weeks) and each two-week interval of the loaded period (12 weeks) around experimental molars.*

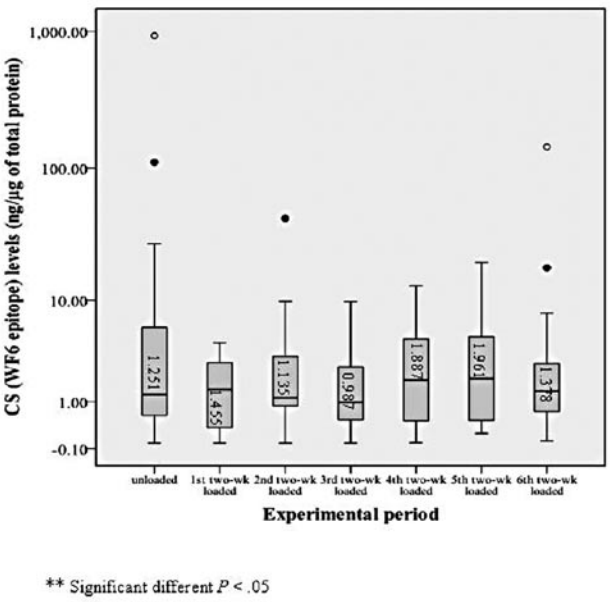

Figure 6 *Boxplot graphs of median CS (WF6 epitope) levels during the unloaded period (2 weeks) and each two-week interval of the loaded period (12 weeks) around control right mandibular first molars.*

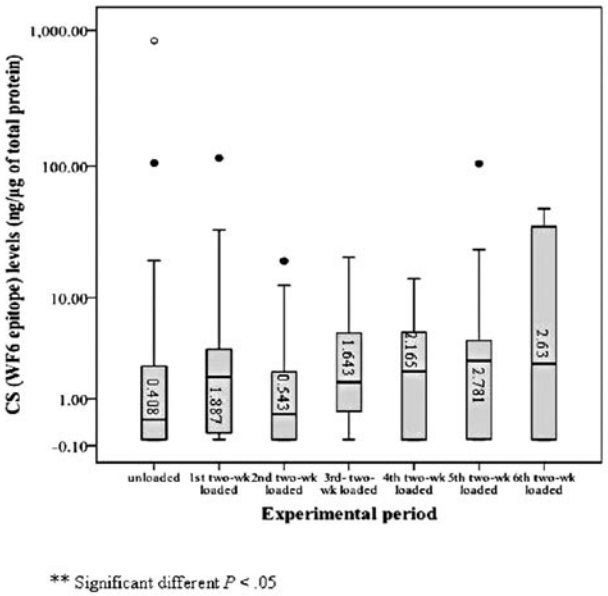

Figure 7 *Boxplot graphs of median CS (WF6 epitope) levels during the unloaded period (2 weeks) and each two-week interval of the loaded period (12 weeks) around miniscrew implants.*

serve as a marker for active alveolar bone and periodontal ligament turnover and have been used to investigate alveolar bone remodeling as a result of periodontal disease and orthodontic tooth movement.[7-8,10] A previous study suggested that the CS component in GCF was associated with some clinical conditions, such as untreated chronic periodontitis, healing after periodontal surgery, trauma from occlusion, and orthodontic tooth movement in which degradation of alveolar bone and periodontal ligament occurs.[14] For dental implants, CS levels in peri-implant crevicular fluid have also been used for monitoring bone resorption and health status of dental implants.[15-18]

Previous studies have demonstrated that CS (WF6 epitope) could be detected in both GCF and PMICF by using ELISA with monoclonal antibody WF6.[10-11] It has been suggested that the concentration of CS (WF6 epitope) in GCF might provide a means for monitoring bone resorption during orthodontic canine movement and that the

GCF (WF6 epitope) levels in PMICF might be associated with bone resorption around miniscrew implants.

In this study, the CS (WF6 epitope) levels in GCF and PMICF were investigated in a manner similar to those used for GCF and PMICF in previous studies.[10-11] CS (WF6 epitope) was detected in human GCF and PMICF samples collected around experimental molars undergoing orthodontic intrusion, around control molars, as well as around miniscrew implants.

The results showed that the median CS (WF6 epitope) levels during the loaded period (12 weeks) around the intruded experimental molars was significantly greater than those during the unloaded period (2 weeks) ($P<.05$). These findings coincided with those of Samuel et al.[19] and Baldwin et al.[20] in which the vertical component of tooth movement produced an increase of CS levels. However, those previous studies quantified the CS levels (in GCF of orthodontically moved canines) by using electrophoresis. This electrophoresis method is a lengthy procedure and requires manipulations of the sample. Therefore, it is not suitable as a quick chair-side method for glycosaminoglycan quantification.

Our results are also similar to those of Last et al.[9] and Kagayama et al.,[21] who reported an increase in CS levels in GCF at the compression side of the tooth during active orthodontic movement. In addition, our findings also correspond to those of Jaito et al.,[10] who reported that there was an increase in CS (WF6 epitope) levels in GCF around canines undergoing orthodontic movement, and that the CS (WF6 epitope) levels in GCF around incisors, which served as control teeth, were not increased.

The increase of CS (WF6 epitope) levels in our study may be explained as follows. Since the apical third of the root is the zone of main pressure in intrusion movement, mechanical stress may alter blood flow and trigger cellular degeneration.[22] Connective tissue breakdown causes a release of glycosaminoglycans into GCF.[23] The relatively high concentration of CS in human alveolar bone (94%) suggests that the alveolar bone may be the main source of CS in GCF.[18] Thus, perturbation of alveolar bone during orthodontic tooth movement may enhance the amount of CS found in GCF.[18] Therefore, it is likely that the significant increase of CS (WF6 epitope) levels in GCF around the intruded experimental molars in our study may result from a degradative process of the extracellular matrix of the alveolar bone during the application of an intrusion force. However, root resorption should also be monitored during studies such as this.

For better understanding of bone resorption around orthodontically moved teeth, we compared the median CS (WF6 epitope) levels around the experimental molars during the unloaded period (2 weeks) with those during each two-week interval of the loaded period. Statistically significant differences were found among the medians of CS (WF6 epitope) levels during the unloaded period (2 weeks) and those during each two-week intervals of the loaded period (12 weeks) around the experimental molars ($P<.05$). On the other hand, no statistically significant difference was found around control molar teeth or around miniscrew implants. This finding may suggest that the monoclonal antibody WF6 can detect CS (WF6 epitope) in GCF and in PMICF. CS (WF6 epitope) may serve as a biochemical marker of alveolar bone turnover within the first two-week interval of orthodontic loading, and may be used as a chair-side diagnostic tool during clinical orthodontic practice in the future. However, the results from this study should be interpreted carefully because a small sample size was used.

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