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The correlation between the bone probing, radiographic and histometric measurements of bone level after regenerative surgery

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Background and objective: The most accurate method of assessing bone level is to elevate the flap and measure the bone level directly. However, this method causes discomfort to the patient and can damage the tissues. Therefore, many studies have been conducted to find an alternative method that can be used to assess the bone level clinically with accuracy and reliability. In the present study, we evaluated the clinical reliability and accuracy of bone probing and radiographic measurements, by comparing the bone levels obtained by both of these measurement techniques with the histometrically confirmed bone levels, after four different kinds of regenerative therapy.

Methods: Twenty-four intrabony defects (4×4 mm one-wall intrabony defects) were surgically created bilaterally in the mandibular second and fourth premolars of six beagle dogs. The control group underwent a conventional flap operation. The graft group was treated with calcium phosphate glass only, the guided tissue regeneration group was treated with guided tissue regeneration only, and the graft + guided tissue regeneration group was treated with calcium phosphate glass and guided tissue regeneration. Bone probing and radiographic measurements were performed to assess the bone level 8 weeks after the operation and then the subjects were killed to perform the histometric measurements. The correlation between the bone probing depths and the histometric bone levels, and that between the radiographic bone levels and the histometric bone levels were analyzed by Spearman's rank correlation analysis. The statistical significance with respect to the type of regenerative therapy was analyzed by the Kruskal–Wallis test.

Results: The difference between the bone probing depth and the histometric bone level measurements was 0.14, and that between the radiographic bone level and histometric bone level was 0.6. The coefficient of correlation between the bone probing depth and the histometric bone level was 0.90, and that between the radiographic bone level and the histometric bone level was 0.73. The type of regenerative therapy had no significant effect on the difference between the histometric bone level and the other measurements.

Conclusion: The results of the present study suggest that the bone probing measurement may be a reliable method for the assessment of the actual bone level following any type of periodontal regenerative therapy.

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The ultimate goal of periodontal therapy is not only to stop the periodontal disease, but also to regenerate the destroyed periodontal tissue and enable it to function normally. The regeneration of periodontal tissue involves the formation of new cementum, the functional insertion of periodontal ligament fiber into it, and the subsequent increase in the bone level. The most frequent operations used for the regeneration of periodontal tissues are the flap operation, bone graft, guided tissue regeneration, and a combination of bone graft and guided tissue regeneration.

In assessing the regenerated periodontal tissue, it is difficult to judge whether true regeneration has taken place without histological evaluation. A number of parameters have been used for the evaluation of the regenerated bone level (1, 2). The most accurate method of assessing the bone level, of course, is to elevate the flap and measure the bone level directly. However, this method causes discomfort to the patient and can damage the regenerated tissues. Therefore, many studies have been conducted to find an alternative method that can be used to assess the bone level clinically with accuracy and reliability (2–7). For example, among the methods that have been proposed so far are the probing depth measurement, various radiographic bone level measurements, and the bone probing technique.

Bone probing measurement requires the insertion of a probe until the tip contacts the bone, a technique that Easley (8) termed the bone sounding technique. In this method, a probe is penetrated horizontally and vertically through the anesthetized gingiva down to the bone in order to assess the bone morphology (8). Greenberg *et al.* referred to this technique as transgingival probing and reported that the vertically probed bone level and the surgically confirmed bone level were closely correlated (3). In subsequent studies, it has been shown that the bone probing measurement is closely correlated with the actual bone level (1, 2).

As regards the radiographic measurement of the bone level, there are several techniques that can be used, including panoramic, bitewing and periapical radiography, and computed tomography. Although the most accurate radiographic measurement method is computed tomography, it is prohibitively expensive (9). Among the commonly used types of clinical radiography, periapical radiography constitutes a more accurate method of assessing the bone level than panoramic or bitewing radiography (10, 11). However, periapical radiography cannot be used to visualize the various forms of bone loss in three dimensions, and causes an image enlargement of up to 4–8% (10, 12). In an attempt to compensate for this, Akesson *et al.* used a stainless steel ball to calculate the enlargement of the radiographs (10), whereas Duckworth *et al.* devised the modified film holder technique (13). In addition, Windisch *et al.* used this modified film holder technique to evaluate the amount of regenerated bone after guided tissue regeneration (14). Kim *et al.* showed that the actual bone level is highly correlated with the bone probing level, but less correlated with the radiographic bone level, and suggested that the bone probing measurement might provide a good clinical method of assessing the bone level following periodontal treatment (1). However, in this study, the bone level was not assessed after the actual regenerative therapy. Also, neither histological evaluation, nor correction for enlargement in the case of the radiographic measurements was done.

In the present study, we performed an assessment of different bone level measurement techniques after periodontal regenerative therapy, including bone probing, radiographic measurement, and histological evaluation (histometric measurement). We enhanced the correlation between the radiographic measurement and the histometric measurement by compensating for image enlargement using the modified film holder technique.

The purpose of the present study is to define the relationship between the measurements obtained by bone probing, radiographic measurement, and histometric measurement when these techniques are used to assess the bone level after performing the conventional flap operation, bone graft, guided tissue regeneration, and a combination of bone graft and guided tissue regeneration. We also studied the difference in reliability and accuracy of each assessment method according to the type of surgery performed.

Material and methods

Six 2-year-old male beagle dogs, weighing about 15 kg, were used for the experiment. All of their teeth were fully erupted and the periodontal tissues were in a healthy state. The institution Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (having achieved AAALAC full accreditation) approved the selection of the animals, management, surgical protocol, and preparation routines. The animals were fed a soft diet throughout the study to reduce the chance of mechanical interference with healing during food intake.

We used a newly manufactured form of calcium phosphate as the bone substitute. Calcium phosphate glass with a Ca/P ratio of 0.6 was prepared from the CaO–CaF_2–P_2O_5–MgO–ZnO system. The particle size of the powdered sample was found to be 200–500 μm. In addition, we used a resorbable membrane (Resolut®, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) for the guided tissue regeneration procedure (15, 16). For the bone probing measurement, we used color-coded probes (Hu-Friedy, Chicago, IL, USA) calibrated up to 15 mm in 1-mm intervals.

Surgical protocol

The surgical procedure was performed under general anesthesia induced by an intravenous injection of atrophine and an intramuscular injection with a mixture of xylazin and ketamine, followed by the inhalation of enfluran. Routine dental infiltration anesthesia (2% lidocaine HCl) was used at the surgical site. Both mandibular third premolars were extracted prior to the experimental surgery, and the extraction sites were allowed to heal for 2 months. After 2 months, mesio-distal width 4 mm × height 4 mm one-wall

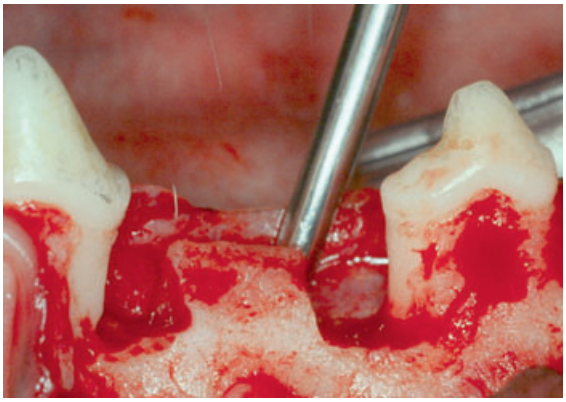

Fig. 1. Surgically created one-wall defect.

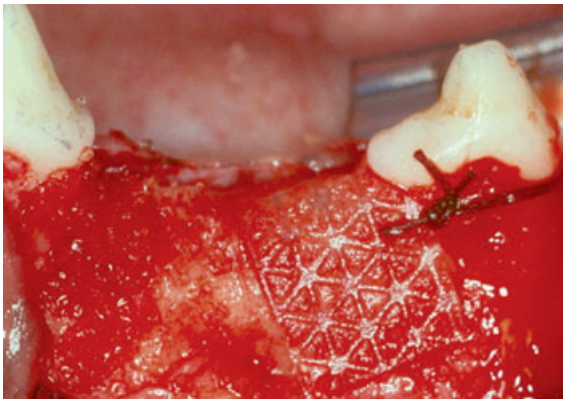

Fig. 2. Calcium phosphate graft and guided tissue regeneration.

intrabony defects were surgically created on the distal side of the second premolar and the mesial side of the fourth premolar under the same general and local anesthesia (Fig. 1). A total of 24 surgical sites were involved for the six beagle dogs (six sites per group). Following thorough root planing, a reference notch was made with a 1/4 round bur on the root surface at the base of the defect. The 24 bilateral intrabony defects were randomly assigned into four groups. The control group underwent a conventional flap operation. The graft group was treated with calcium phosphate glass only, the guided tissue regeneration group was treated with guided tissue regeneration only, and the graft + guided tissue regeneration group was treated with calcium phosphate glass and guided tissue regeneration (Fig. 2). The flaps were repositioned and sutured with 4–0 coated Vicryl® (Ethicon Ltd, Edinburgh, UK). The sutures were removed after 14 days. Postsurgery management included the administration of antibiotics intramuscularly (Tetracycline HCl), a soft diet and the daily topical application of a 0.12% chlorhexidine solution (Hexamedin, Bukwang Pharmaceuticals Co., Seoul, Korea).

Radiographic measurement procedure

At 8 weeks after the first surgical procedure, standardized periapical radiographs were obtained using modified film holders prior to killing the animals. Horizontal wire, rectangular wire and round wire were placed on the film holder at a specified position (Fig. 3). The images of these wires were obtained on the radiographs (Fig. 4). For the radiographic measurement, we digitalized each radiograph using a Photo smart S20® scanner (Hewlett Packard Co., Palo Alto, CA, USA). We observed the distance from the cementoenamel junction to the coronal extension of the newly formed alveolar bone along the root surface using a PC based image analysis system (Image Pro Plus, Media Cybernetics, Silver Spring, MD, USA).

Bone probing measurement procedure

Prior to killing the animals at 8 weeks following the first surgical procedure,

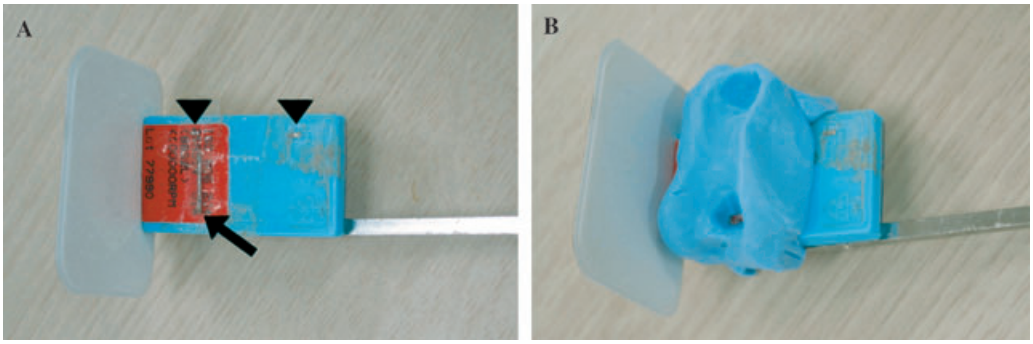

Fig. 3. (A) Modification to Extension Cone Paralleling instrument (XCP, Rinn Co., Elgin, IL, USA). Horizontal wire (arrow) and coaxial wires (arrowheads: one square, the other round) on bite surface. (B) Poly ether (Aquasil® soft putty, Dentsply, Germany) occlusal registration.

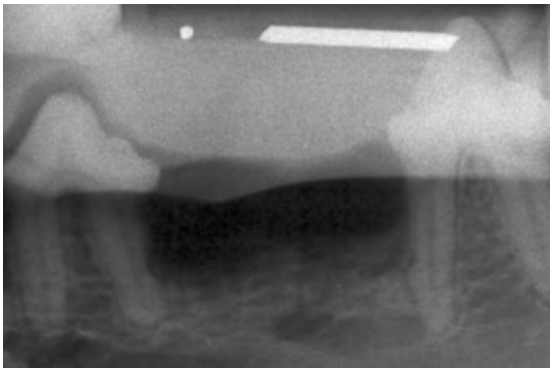

Fig. 4. Radiograph taken with the modified film holder technique.

we performed bone probing in the direction of the long axis along the proximal surface of the teeth under local anesthesia. The reference point was the cementoenamel junction. The depth at which the probe met strong resistance from the bone was recorded as the bone probing depth.

Histometric measurement procedure

The animals were killed 8 weeks after the first surgical procedure by means of an intravenous injection of concentrated sodium pentobarbital. In many studies (17–19), it has been confirmed that an 8-week healing interval is required to evaluate the bone regenerating capability of space-maintaining biomaterials. Block sections including the surgical sites were removed, rinsed in saline, and fixed in 10% buffered formalin. Subsequently, the block sections were decalcified in 5% nitric acid and embedded in paraffin. Then 5-μm thick serial sections were made in the mesiodistal direction. The four most central sections from each block were stained with hematoxylin and eosin. For the histometric measurement, the cementoenamel junction and the notch were used as reference points (Fig. 5). We measured the distance from the cementoenamel junction to the coronal extension of the newly formed alveolar bone along the root surface using a PC-based image analysis system (Image Pro Plus, Media Cybernetics). The histometric measurements from the four sections in each block were used to calculate the mean score.

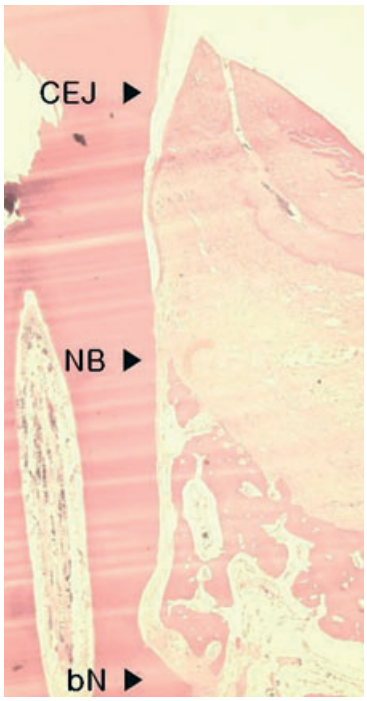

Fig. 5. Section of operation site showing a coronally grown new bone. CEJ: cementoenamel junction; NB: new bone regeneration; bN: base of the reference notch.

Statistical analysis

The correlation between the bone probing depths and the histometric bone levels, and that between the radiographic bone levels and the histometric bone levels were analyzed with Spearman's rank correlation analysis. The statistical significance with respect to the type of regenerative therapy was analyzed with the Kruskal–Wallis test.

Results

Clinical measurements

The mean bone probing depth was 3.13 ± 0.68 mm for all sites, with the individual group measurements being 3.33 ± 0.82 mm for the control group, 2.83 ± 0.75 mm for the graft group, 3.33 ± 0.52 for the guided tissue regeneration group, and 3.00 ± 0.63 mm for the graft + -guided tissue regeneration group. The mean radiographic bone level (RBL) was 3.87 ± 0.88 mm for all sites, with the individual group measurements being 3.70 ± 0.51 mm, 3.52 ± 0.87 mm, 4.15 ± 1.17 mm, and 4.12 ± 0.91 mm for the control, graft, guided tissue regeneration, and graft + guided tissue regeneration groups, respectively. The mean histometric bone level was 3.27 ± 0.5 mm for all sites, with the individual group measurements being 3.32 ± 0.58 mm, 3.12 ± 0.52 mm, 3.43 ± 0.42 mm, and 3.20 ± 0.53 mm for the control, graft, guided tissue regeneration and graft + guided tissue regeneration groups, respectively (Table 1).

Correlation between the measurements

For all sites, the coefficient of correlation between the bone probing depth and the histometric bone level was 0.90, and that between the radiographic bone level and the histometric bone level was 0.73 (Table 1, Fig. 6). For the control group, the coefficient of correlation between the bone probing depth and the histometric bone level was 0.93, and that between the radiographic bone level and the histometric bone level was 0.60 (Table 1, Fig. 7). For the graft group, the coefficient of correlation between the bone probing depth and the histometric bone level was 0.94, and that between the radiographic bone level and the histometric bone level was 0.58 (Table 1, Fig. 8). For the guided tissue regeneration group, the coefficient of correlation between the bone probing depth and the histometric bone level was 0.83, and that between the radiographic bone level and the

Table 1. Mean value and standard deviation for each clinical measurement for regenerative therapies

Group	BPD	RBL	HBL	HBL-BPD	HBL-RBL	γ (BPD:HBL)	γ (RBL:HBL)
All site	3.13 ± 0.68	3.87 ± 0.88	3.27 ± 0.5	0.14 ± 0.31	−0.6 ± 0.62	0.90	0.73
Control	3.33 ± 0.82	3.70 ± 0.51	3.32 ± 0.58	−0.01 ± 0.28	−0.38 ± 0.41	0.93	0.60
Graft	2.83 ± 0.75	3.52 ± 0.87	3.12 ± 0.52	0.29 ± 0.38	−0.39 ± 0.56	0.94	0.58
GTR	3.33 ± 0.52	4.15 ± 1.17	3.43 ± 0.42	0.10 ± 0.19	−0.72 ± 0.83	0.83	0.70
Graft + GTR	3.00 ± 0.63	4.12 ± 0.91	3.20 ± 0.53	0.20 ± 0.33	−0.92 ± 0.62	0.85	0.71
p-value				0.39	0.55		

BPD: bone probing depth; RBL: radiographic bone level; HBL: histometric bone level; GTR, guided tissue regeneration.
$n = 6$ per group (all site: $n = 24$).
Spearman's correlation value (γ).
Statistical significance, $p < 0.05$.

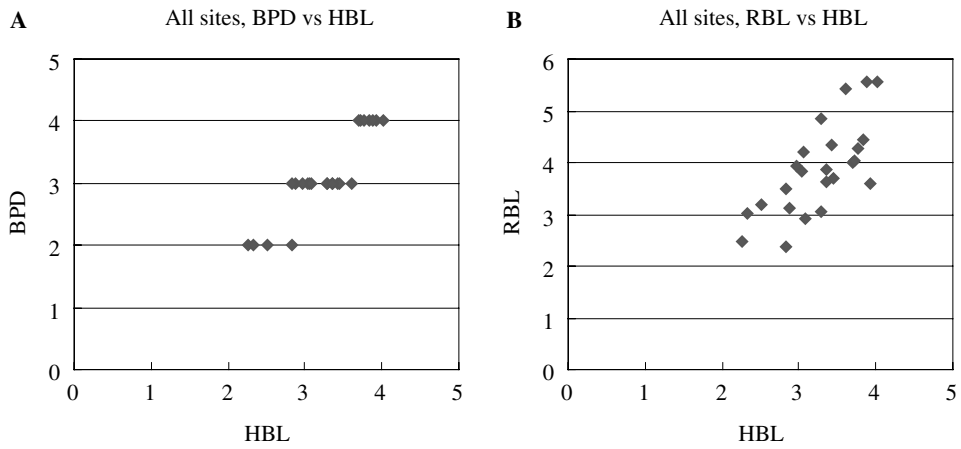

Fig. 6. (A) Scattergram showing correlation between bone probing depth (BPD) and histometric bone level (HBL) for all sites (γ = 0.90). (B) Scattergram showing correlation between radiographic bone level (RBL) and histometric bone level (HBL) for all sites (γ = 0.73).

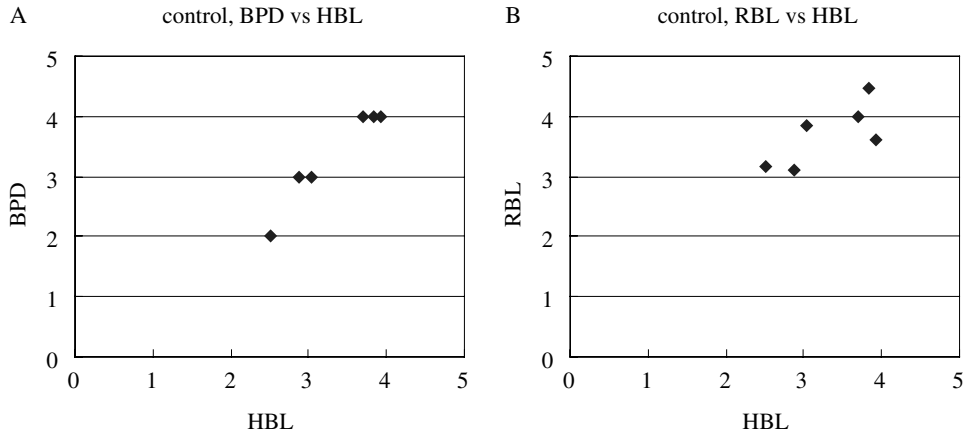

Fig. 7. (A) Scattergram showing correlation between bone probing depth (BPD) and histometric bone level (HBL) for the control group (γ = 0.93) (B) Scattergram showing correlation between radiographic bone level (RBL) and histometric bone level (HBL) for the control group (γ = 0.60).

histometric bone level was 0.70 (Table 1, Fig. 9). For the graft + guided tissue regeneration group, the coefficient of correlation between the bone probing depth and the histometric bone level was 0.85, and that between the radiographic bone level and the histometric bone level was 0.71 (Table 1, Fig. 10).

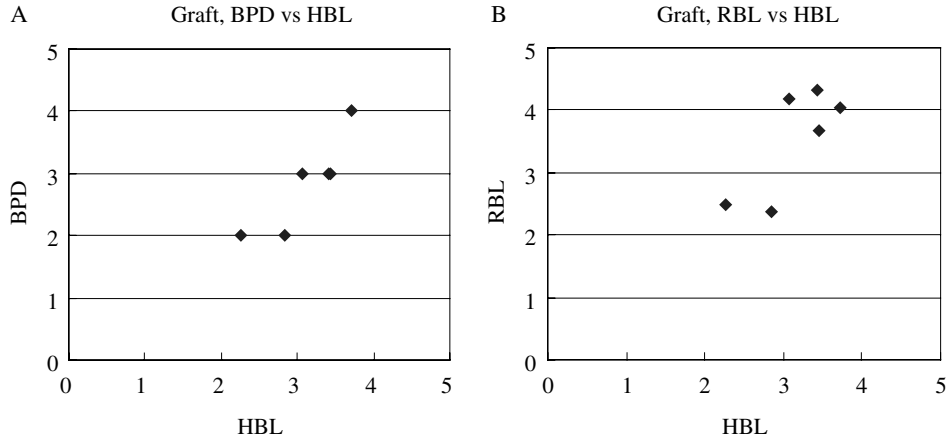

Fig. 8. (A) Scattergram showing correlation between bone probing depth (BPD) and histometric bone level (HBL) for graft group ($\gamma = 0.94$) (B) Scattergram showing correlation between radiographic bone level (RBL) and histometric bone level (HBL) for the graft group ($\gamma = 0.58$).

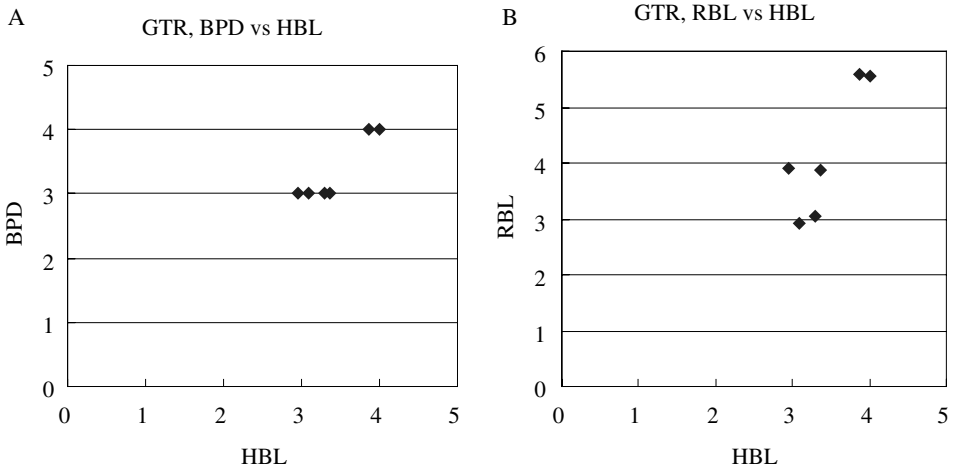

Fig. 9. (A) Scattergram showing correlation between bone probing depth (BPD) and histometric bone level (HBL) for the guided tissue regeneration group ($\gamma = 0.83$) (B) Scattergram showing correlation between radiographic bone level (RBL) and histometric bone level (HBL) for the guided tissue regeneration group ($\gamma = 0.70$).

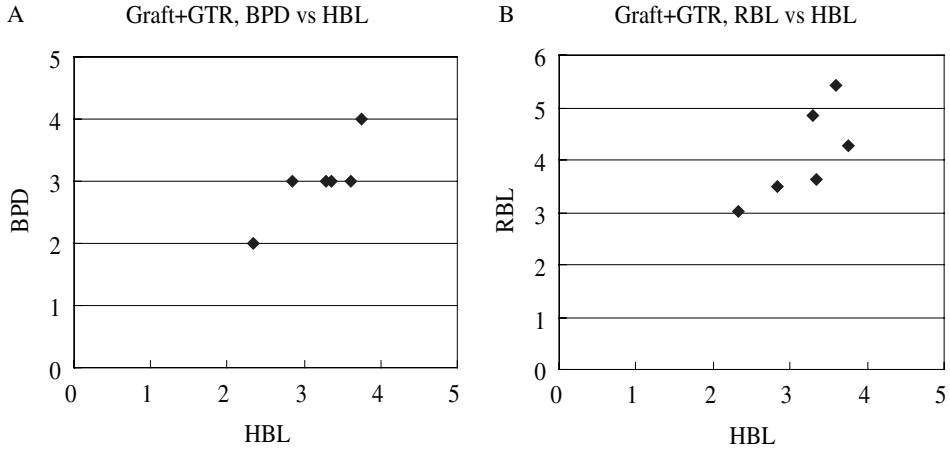

Fig. 10. (A) Scattergram showing correlation between bone probing depth (BPD) and histometric bone level (HBL) for the graft + guided tissue regeneration group ($\gamma = 0.85$) (B) Scattergram showing correlation between radiographic bone level (RBL) and histometric bone level (HBL) for guided tissue regeneration group ($\gamma = 0.71$).

The difference between the measurements with respect to the type of regenerative therapy

For all sites, the difference between the bone probing depth and the histometric bone level was 0.14 ± 0.31 mm and that between the radiographic bone level and the histometric bone level was −0.60 ± 0.62 mm. For the control group, the difference between the bone probing depth and the histometric bone level was 0.01 ± 0.28 mm and that between the radiographic bone level and the histometric bone level was −0.38 ± 0.41 mm. For the graft group, the difference between the bone probing depth and the histometric bone level was 0.29 ± 0.38 mm and that between the radiographic bone level and the histometric bone level was −0.39 ± 0.56 mm. For the guided tissue regeneration group, the difference between the bone probing depth and the histometric bone level was 0.10 ± 0.19 mm and that between the radiographic bone level and the histometric bone level was −0.72 ± 0.83 mm. For the graft + guided tissue regeneration group, the difference between the bone probing depth and the histometric bone level was 0.20 ± 0.33 mm and that between the radiographic bone level and the histometric bone level was −0.92 ± 0.62 mm (Table 1).

There was no statistically significant difference ($p > 0.05$) either between the bone probing depth and the histometric bone level or between the radiographic bone level and the histometric bone level with respect to the type of regenerative therapy.

Discussion

We surgically created one-wall intrabony defects on the proximal side of the premolar of the beagle dogs. To enhance the accuracy of the radiographs, we simplified the intrabony defects so that they had only one wall. This is based on a report that there is no difference in the healing response between surgically created defects and natural periodontal defects (20). This type of surgically formed defect also makes it possible to assess the regenerated bone more efficiently, by providing similar initial experimental conditions for both the experimental and control groups (21). The calcium phosphate used in this experiment is a stable and efficient synthesized graft material for intrabony defect treatment, and is used mainly in two forms: hydroxyapatite and tricalcium phosphate (22, 23). The absorbable barrier membranes consisting of a copolymer of polylactic acid and polyglycolic acid, which are used in guided tissue regeneration, were developed several years ago and their excellent quality and stability have previously been reported (15, 16).

The main objective of this study was to evaluate the clinical reliability and accuracy of the bone probing measurement, radiographic measurement, and histometric measurement in the assessment of the bone level following various regenerative therapies. There are many studies involving bone probing that compare the bone probing level with the actual bone level after the elevation of the flap. Kim *et al*. reported a difference of 0.02 mm and a coefficient of correlation of 0.92 between the bone probing level and the actual bone level (1). In similar studies, Renvert *et al*. reported a difference of 0.3 mm and a coefficient of correlation of 0.81 between the bone probing level and the actual bone level (2), and Ursell reported a difference of 0.12 mm and a coefficient of correlation of 0.975 between the bone probing level and the actual bone level (7).

In the present study, we compared the bone probing measurement, radiographic measurement and the histometric measurement after the different regenerative therapies in order to evaluate the bone level more accurately. From our results, the difference between the bone probing measurement and the histometric measurement was 0.14 mm with a very high correlation ($\gamma = 0.90$). On the other hand, the difference between the radiographic measurement and histometric measurement was 0.6 mm and the coefficient of correlation (γ) was 0.73. In a previous study, Kim *et al*. reported a difference of 0.57 mm and a coefficient of correlation of 0.68 between the bone probing level and the radiographic bone level (1). It seems that the slightly high coefficient of correlation of the present study is due to the use of the modified film holder technique, which is assumed to compensate for the enlargement of the image. There were no statistically significant differences between the measurements obtained after the conventional flap operation, bone graft, guided tissue regeneration, and the combination of bone graft and guided tissue regeneration, all of which are typically used for regenerative therapy. In previous studies (2, 14), it was reported that the most accurate method of assessing the bone level is histometric measurement. Therefore, by comparing the bone probing and radiographic measurement with histometric measurement in the present study, we are able to conclude that bone probing constitutes a more reliable method of bone level assessment than radiographic measurement, regardless of the type of regenerative therapy performed.

In many studies, it has been reported that there is a tendency for the radiographic method to underestimate the bone level compared to the histometric method, which gives the actual bone level (6, 12). There are several possible reasons for this. First, regenerated immature bone cannot be seen on the radiographic image. Second, as Moon *et al*. (24) and Barney *et al*. (25) have reported, the regeneration pattern of newly developed bone, which follows the newly formed cementum, grows coronally, so that the thin part of the regenerated bone cannot be seen on the radiographic image. In the present study, the radiographic bone level was approximately 0.6 mm shorter than the histometric bone level. Based on these data, we can assume that although the bone was histologically regenerated at 8 weeks after the regenerative therapies, the upper 0.6 mm part of the bone had not yet matured sufficiently to be seen on the radiographic image.

In conclusion, our results suggest that bone probing measurement constitutes a reliable method for the assessment of the actual bone level regardless of the method of regenerative therapy employed. Therefore, the

bone probing measurement may provide a good clinical method of assessing the regenerated bone level after periodontal therapy.

Acknowledgements

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