Detecting bone changes along dental implants, after immediate or delayed loading, using digital subtraction on cropped panoramic radiographs. A prospective clinical trial with minimum 3-year follow up

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ABSTRACT

Aim The aim of this study was to assess bone changes along implants after immediate or delayed loading, using subtractions of digital images originated from cropped panoramic radiographs and visual evaluation.

Materials and methods Eleven patients received 4 Ankylos implants interforaminal in the mandible. In 7 patients the implants were loaded immediately and 4 followed delayed loading. All patients were restored with a telescopic overdenture with Syncone abutments. From each patient 3 panoramic radiographs (PRs) were obtained: upon delivery of the restoration (T1), 6 months later (T2) and after 3 years (T3). 33 implants were finally selected. The radiographs were analyzed using the Emago[®] Software. The grey scale values were measured either manually (Stage A) or automatically (Stage B) in six areas (neck, middle and apex; both mesially and distally) along the implants' sides to evaluate the bone density during clinical function. Images were also visually evaluated by five observers to detect bone changes at the cervical implant area.

Results Strong positive correlation between the two stages (A and B) was found in all 3 examinations (Pearson's r 0.84-0.98). The t-test showed no statistically significant differences in grey level values between immediate and delayed loading (p<0.05) and no statistically significant changes in the visual evaluation among implants undergoing either immediately or delayed loading (p<0.05).

Conclusions Emago[®] is a valuable method for bone level assessment around implants' neck. The grey value measurements of the bone adjacent to the implants that have been loaded either immediately or delayed do not significantly differ after 3 years of fuction. The visual assessment of the PRs images supports these findings.

KEYWORDS Bone change, Digital image, Grey value, Immediate loading, Subtraction radiographs.

INTRODUCTION

Nowadays, root-shaped dental implants represent a highly recognized method for a successful outcome in oral rehabilitation. Regarding their prognosis, consensus was reached by consolidating the terminologies of implants' success, survival and loss (1,2,3). Conventionally, the loading protocol indicates that the prosthetic rehabilitation is constructed and delivered after 3-6 months. On the other hand, the immediate loading protocol enables the insertion of the prosthetic rehabilitation, in occlusion with the opposite arch, within 48h (4). Numerous studies have reported the success rates for conventional and immediate loading protocols (5-16). Yet, only few studies have evaluated the bone loss along dental implants, after immediate and delayed loading, using subtraction images. It is widely known that both clinical examination and radiographic evaluation are the basic procedures used to diagnose bone loss around an implant and especially around its neck. However, it still remains uncertain whether dentists can recognize minimal changes of bone, as of 0.02 mm that is considered a normal annual bone loss after the first year of loading (17,18,19) using either conventional periapical or panoramic radiographs (PRs). Since its introduction in the dental practice in 1980, digital subtraction radiography (DSR) has proven to be a sensitive method for the detection of changes in tissues' mineral content (20). The hypothesis supports the concept that images of unchanged structures are cancelled when they are digitally superimposed and subtracted. Then, any differences can be easily recognized. On the contrary, in conventional radiographs bone changes can be visually evaluated only after 30-40% mineral loss, indicating that minor

changes cannot be detected using those radiographs (21,22). DSR was first used in periodontology to identify small changes of crestal bone after periodontal therapy (23-26). Only few studies have examined changes around implants after immediate and delayed loading using DSR (27).

Agreement between the DSR results and visual evaluation with regards to bone changes around the implants' cervical area has never been investigated so far, whereas the present study combines digital images produced by a DSR software and visual evaluation by 5 observers.

Aim

This study primarily aims to indirectly assess bone changes which occurred along implants, after immediate or delayed loading and were expressed as grey value changes of digitally subtracted images from cropped PRs and subsequently to correlate the results with those of the visual assessment.

MATERIALS AND METHODS

Sample selection criteria (patients and implants)

Eleven patients who participated in a prospective clinical trial of the Dental School of the University of Athens were retrospectively evaluated. There were 4 males and 7 females, 4 were smokers and 7 non-smokers. The patients' age ranged from 53 to 74 yrs. (Mean age = 65 yrs.; SD=7.21yrs). Seven patients (carrying 28 implants) received immediate loading and 4 (carrying 16 implants) delayed loading. All patients selected were following a strict recall program which also included PRs that had to be taken at specific time intervals.

Both the clinical trial and the present research were approved by the Ethical Committee of the Dental School of National and Kapodistrian University of Athens, Greece. The patients were informed and undersigned written consent in order to participate in the current study.

All patientsreceived 4 Ankylos implants (Dentsply Implants GmbH, Mannheim, Germany) 9-13 mm in length and 3.5 mm diameter which were inserted interforaminally in the mandible and were restored with an overdenture using Ankylos Syncone system (Dentsply Implants GmbH, Mannheim, Germany). The rehabilitation protocol required prefabricated conical abutments fixed on implants by a fixing screw and an overdenture placement which was supported and retained on the abutments by prefabricated gold telescope copings. All patients had either a conventional complete denture or a fixed rehabilitation in the maxilla. In this clinical trial the implants were loaded immediately taking under consideration the adequate primary stability (>40 Ncm) which could be reached intrasurgically. Alternatively, a conventional loading protocol was followed and the patients received the same kind of restoration 3 months later.





FIG. 1 Grey scale mode of the cropped anterior region of the mandible.

FIG. 2 Cropped image of an implant.

Radiographic examination and processing

Three PRs were obtained from each patient: the first upon the delivery of the restoration (T1), the second 6 months later (T2) and the third 3 years later (T3). Out of a total of 44 implants that were initially included in this study, 11 were excluded due to distortion which appeared in the interforaminal region of one or more radiographs. Finally, 33 implants constituted the cohort of our study. The patients' PRs were digitized using a dental camera (Canon EOS 1100D, macro 100mm lens without ring flash) at a LED lightbox. All digital images were obtained from a standardized distance with a standard frame and standardized shutter and time of exposure. The images were converted into grey scale mode, resolution of 300dpi, and resized to 15x10cm format using a photo processing software (Adobe Photoshop 7.0). Each digital image was subjected to further manipulation; the area above the mandible was cropped (Fig. 1) and then each depicted implant was recropped separately to pictures sized 2x4cm and stored as a non-compressed Tiff file format (Fig. 2). Thus, 3 images were created for each implant, T1, T2 and T3, which corresponded to the time of recall.

Image evaluation

The cropped digital images of each implant were evaluated using two different procedures: a DSR software Emago[®] (Oral Diagnostics System, Amsterdam, The Netherlands) and visual assessment as follows:

 The cropped images were compared using Emago[®] in two different stages (stage A and B), which are analytically presented below. It should be underlined that both stages (A and B) measure the difference of grey level values in specific areas of interest along the implants' sides. In stage A, the difference on the reconstructed images was calculated by the investigator (ES) comparing the grey values of each specific area for all implants. In stage B, the aforementioned grey value differences were calculated on the subtracted image by the software. The two stages procedure was followed in order to verify the reliability and reproducibility of the software.

2) The images were visually evaluated by 5 experienced clinicians and oral radiologists to detect bone loss around the implants' cervical areas.

Adjusting grey levels and geometry

All images included in this study had been modified, as to the grey value level, and geometrically registered using Emago[®]. For each implant examined, six regions of interest (ROI) sized 5x5mm were selected on the periimplant bone, 1 mm from the implant surface to avoid contamination (beam hardening effect) of the metallic surface of the implant. In order to ensure the reliability of the method and to standardize the size of the ROI, an identical 5x5mm area corresponding to the internal fabricated part of the implants' digital image was selected and considered as an internal control and standard. ROIs were applied at six areas around the implant: mesial neck, mesial middle, mesial apex, distal neck, distal middle and distal apex. Then, a two-stage procedure was followed.

Stage A (calculating grey value difference)

First, once images were submitted to Emago[®], the grey scale values distribution was adjusted for all T1, T2 and T3 digital images and then matched to each other. This was performed using the "gamma correction" command of the software (Fig. 3). Thereafter, the images were "reconstructed" and minimal geometric differences between radiographs of the depicted implants were corrected. 4 reference points mesial and distal (for both the neck and the apex area) were selected on each of the examined implants and geometric registration was held (Fig. 4). These reference points are referred as "landmarks" in the software. Due to the lack of standardization of the radiographs, the images' "reconstruction" was performed for each implant separately. Thus, an accurate superimposition and comparison of the radiographic images was reached. At this stage, the grey values for each of the aforementioned 6 areas around the examined implants were calculated. Each of the T1 measurements was subtracted from the T2, the T2 from the T3 and the T1 from the T3 respectively.

Stage B (grey value estimation in subtracted images)

After the grey scale correction and geometric reconstruction of the 3 digital images of each implant (now T1Rec, T2Rec and T3Rec) digital subtraction of the images followed. Four reference points, mesial and distal for both the neck and the apex accordingly, were selected. At this stage any gray value difference was calculated by subtraction of the grey values of the first subtracted image (T1Rec) from those of the second image (T2Rec), the second (T2Rec) from the third (T3Rec) and the first (T1Rec) from the third image (T3Rec).

The resulted positive or negative numbers were also recorded. Overall, 99 images were produced and stored as Tiff files format (Fig. 5, 6).



FIG. 3 Greyscale matching between T1 and T2.



FIG. 4 Geometric registration between T1 and T2 recall, using 4 landmarks at each implant.



FIG. 5 The subtraction procedure between T1Rec and T2Rec images using the 4 reference points.



FIG. 6 Subtraction image (the third in the row from left).



FIG. 7 Measurement of grey value at the mesial neck area of the T2Rec digital image.



FIG. 8 Measurement of grey value at the mesial neck area of the subtraction image.

Following the two-stage procedure the accuracy of our measurements was ensured regarding the intraevaluation reliability.

Grey values assessment

Emago[®] was also used to assess grey values on the processed T1Rec, T2Rec, T3Rec images (Fig. 7) and on the subtraction images as previously described (Fig 8). Grey scale values normally ranged from 0 to 255 (Emago[®] supports 8 bits image files format). In the ROIs of peri-implant areas, grey values above 128 indicate mineral gain and below 128 mineral loss (Carneiro et al 2012). Due to the great clinical significance of the cervical region, the mesial and the distal area of the neck was calculated separately. For the other areas (middle and apex) the mesial and distal area values were merged as a single mean.

Visual examination

The same pool of cropped PRs' images that were used by Emago[®] was also visually evaluated by 5 experienced observers. The visual comparison was performed in order to estimate the bone changes at the implants' neck with naked eye. More specifically, it was of interest to identify first whether the clinician is able to detect small or minimal differences at the cervical bone level using PRs, secondly if the assessment of the 5 observers shows cohesion and, finally, if there is a correlation between the results of their estimation and the applied loading protocol.

The evaluation procedure that was followed by the 5 observers was identical and included both the implant number and the ROIs (e.g.: #44M: implant at the position 44, region of interest mesial neck). Bone loss or gain was measured at a -3 to + 3 mm scale.

Data analysis

The statistical evaluation was performed with the use of: IBM-SPSS 22, Microsoft Excel 2013 and Statistica 10 Enterprise (Part of Dell Software since 2015). For the reliability of the observational quality the Intra-Class Correlation was used with the consistency being the main criterion. Data were subjected to mean comparison (t-test) at the 0.5 significance level. The Pearson's Correlation Index (r) was also calculated and compared.

RESULTS

Stage A

The results of stage A are shown in Table 1. Positive numbers indicate increased grey level values and negative numbers lowered.

Stage B

The results of stage B are shown in Table 2. Data from both stages (A and B) are presented in Figure

ROI	D 1-2 (1st-2n	d Examination)	D1-3 (1st-3rd	Examination)	D2-3 (2nd- 3rd Examination)		
	Mean	Std. Deviation	Mean Std. Deviation		Mean	Std. Deviation	
Mesial_Neck	-4.43	33.42	-9.12	33.53	-4.69	22.98	
Distal_Neck	-2.47	32.67	2.30	29.98	4.77	29.04	
Middle	7.85	21.43	1.73	24.16	-6.11	19.26	
Арех	9.80	27.82	5.28	28.64	-4.52	20.75	

TABLE 1 Mean and Standard Deviation of the differences (D) between the grey level values of T1-T2, T2-T3, T1-T3 images as calculated in stage A.

	Delayed		Immediate			Levene's Test				
	Mean	SD	Mean	SD		F	Sig.	t	df	Sig. (2-tailed)
Neels 1.0	4.47	14.00	0.00	26.66	EVA	2.07	0.00	1.61	31.00	0.12
Neck 1-2	4.47	14.69	-8.60		EVnA	3.97	0.06	1.81	30.39	0.08
Nodel 2	1.05	12.00	0.50	20.12	EVA	2.01	0.00	0.94	31.00	0.35
Neck1-3	1.35	12.88	-6.50	28.12	EVnA	3.91	0.06	1.09	28.54	0.29
	-3.12	18.04	2.10	04.50	EVA	1.02	0.22	-0.66	31.00	0.52
Neck 2-3	2-3 -3.12 18.04 2.10 24.56	EVnA	1.02	1.02 0.32	-0.70	30.43	0.49			
Middle 1 0	6.75	21.60	8.56	21.85	EVA	0.01	0.93	-0.23	31.00	0.82
Middle 1-2					EVnA	0.01		-0.23	26.00	0.82
Middle 1-3	4.91	25.88	-0.33	23.42	EVA	0.22	0.63	0.60	31.00	0.55
IVIIUUIE I-3					EVnA	0.23	23 0.03	0.59	23.90	0.56
Middle 2 2	1.0.4	10.01	0.00	10.00	EVA	0.21	0.21 0.65	1.03	31.00	0.31
Middle 2-3	-1.84	19.01	-8.89	19.39	EVnA	0.21		1.03	26.16	0.31
Amov 1 0	4.1.4	05.05	10.40	00.00	EVA	0.40	0.50	-0.94	31.00	0.35
Apex 1-2	4.14	25.35	13.48	29.36	EVnA	0.48		-0.97	28.41	0.34
Amov 1 0	2.00	80 32.04	11.19	25.30	EVA	1 74	74 0.20	-1.50	31.00	0.14
Apex 1-3	-3.80				EVnA	1.74		-1.42	21.47	0.17
Amory 0, 0	-7.94 23.88		0.00	18.75	EVA	0.01	0.05	-0.76	31.00	0.45
Apex 2-3		23.88	-2.29		EVnA	0.91	0.35	-0.72	21.37	0.48

TABLE 2 Mean and Standard Deviation of the grey level values of T1-T2, T2-T3, T1-T3 on subtraction images in stage B.

9. It is obvious that the fluctuation of the differences in grey level values is similar in both stages.

Correlation between stage A and stage B

Further computational research was carried out aiming to ascertain the correlation between grey level values at the six ROIs (mesial neck, distal neck, mesial middle, distal middle, mesial apex, distal apex) in stage A and stage B accordingly.

Pearson's correlation index (r), which represents an index of the linear correlation between two variables, was used. The values of this index vary from -1, indicating absolute negative correlation, to +1, indicating absolute positive



FIG. 9 Differences of grey level values in stage A and B.

correlation. Comparing 6 ROIs in 3 recalls a total of 18 different figures were obtained. Each figure includes different diagrams: the upper horizontal and right side diagram shows the distribution of the variable values. In the central diagram (scattering diagram) the straight diagonal line indicates the correlation among the two stages (A and B) and the interrupted lines represent the confidence interval. Numerous points within this area indicate a strong correlation (Fig. 10).

Figure 10 presents one of the 18 figures (3 recalls, 6 ROIs) showing the correlation between stage A and stage B.

All figures indicate a strong positive correlation between the method A and the Stage B concerning the aforementioned 6 regions of interest (mesial neck, distal neck, mesial middle, distal middle, mesial apex, distal apex) of the 3 examinations T1Rec, T2Rec and T3Rec. The Pearson's r values ranged from 0.84 to 0.98.

Loading as a predictive factor for grey level value changes

The loading protocol (immediate or delayed) was investigated as a predictive factor affecting the grey level changes around implants (especially at the cervical area). Even though a positive correlation between the two stages was documented, both methods were exanimated separately to ensure maximum accuracy.

Stage A: The mean and standard deviation of the



FIG. 10 Correlation factor (Pearson's r) for stage A and B at the mesial neck area.

differences among the subjected images' grey level values, at the 3 ROIs (mesial, middle, apex) between immediate and delayed loading were calculated using T-test and are summarized in Table 3. No statistical difference in grey values between immediate and delayed loading were detected in stage A (p<0.05). Equal Variances Assumed (EVA)/Equal Variances not Assumed (EVnA): depending on the significance of

	Delayed	Delayed		Immediate		Levene's Test				
	Mean	SD	Mean	SD		F	Sig.	t	df	Sig. (2-tailed)
Node 1 2	4 47	14.00	0.00		EVA	2.07	0.00	1.61	31.00	0.12
Neck 1-2	4.47	14.69	-8.60	26.66	EVnA	3.97	0.06	1.81	30.39	0.08
	1.05	10.00	0.50	20.12	EVA	2.01	0.00	0.94	31.00	0.35
Neck 1-3	1.35	12.88	-6.50	28.12	EVnA	3.91	0.06	1.09	28.54	0.29
NLOO	2 12	10.04	0.40	04.50	EVA	1.00	0.22	-0.66	31.00	0.52
Neck 2-3	3 -3.12 18.04 2.10 24.56 EVnA 1.02	1.02	0.32	-0.70	30.43	0.49				
Middle 1.0	0.75	21.60	8.56	21.85	EVA	0.01	0.02	-0.23	31.00	0.82
Middle 1-2	6.75				EVnA	0.01	0.93	-0.23	26.00	0.82
Middle 1 2	4.01	25.00	-0.33	23.42	EVA	0.22	0.00	0.60	31.00	0.55
Middle 1-3	4.91	25.88			EVnA	0.23	0.63	0.59	23.90	0.56
Middle 2.2	1.0.4	10.01	0.00	10.00	EVA	0.01	0.05	1.03	31.00	0.31
Middle 2-3	-1.84	19.01	-8.89	19.39	EVnA	0.21	0.65	1.03	26.16	0.31
Amov: 1. 2	4.1.4	05.05	10.40	00.00	EVA	0.40	0.50	-0.94	31.00	0.35
Apex 1-2	4.14	25.35	13.48	29.36	EVnA	0.48	0.50	-0.97	28.41	0.34
A	2.00	32.04	11.19	25.30	EVA	1 74	0.20	-1.50	31.00	0.14
Apex 1-3	-3.80				EVnA	1.74		-1.42	21.47	0.17
	704	23.88	-2.29	18.75	EVA	0.01	0.05	-0.76	31.00	0.45
Apex 2-3	-7.94				EVnA	0.91	0.35	-0.72	21.37	0.48

TABLE 3 Mean and Standard Deviation of the grey level values in immediate and delayed loading in stage A.

	Delayed	Delayed		Immediate		Levene's	Levene's Test				
	Mean	SD	Mean	SD		F	Sig.	t	df	Sig. (2-tailed)	
Neck_1-2	1 40	12 50		00.04	EVA	F F0	0.02	-1.34	31.00	0.19	
	-1.42	13.50	9.94	28.31	EVnA	5.59	0.02	-1.54	29.00	0.13	
Nook 2.2	E 00	22.42	-4.08	24.00	EVA	0.02	0.96	1.14	31.00	0.26	
Neck_2-3	5.83	.83 23.42	-4.08	24.99	EVnA	0.03	0.86	1.16	27.00	0.26	
No. 1. 1. 0	1.00	10.00	4 75	21.00	EVA	2 12	0.00	-0.70	31.00	0.49	
Neck 1-3	-1.83	16.20	4.75	31.08	EVnA	3.12	3.12 0.09	-0.79	29.93	0.43	
Middle 1.0	0 5 0	21.00	7.20	24.83	EVA	0.20	0.02	-0.16	31.00	0.88	
Middle_1-2 -	-8.58	21.09	-7.28		EVnA	0.26	0.62	-0.16	28.67	0.87	
	1.40	10.00	0.01	10.00	EVA	0.02	0.05	-0.79	31.00	0.43	
Middle_2-3	1.43	19.99	6.91	19.08	EVnA	0.03	0.85	-0.78	24.91	0.44	
	0.07	20.21	0.44	22.02	EVA	0.05		-0.06	31.00	0.95	
Middle_1-3	-0.97	26.21	-0.44	23.93	EVnA	0.25	0.62	-0.06	24.06	0.95	
A	4 47	07.05	10.00	07.04	EVA	0.00	0.02 0.88	0.90	31.00	0.37	
Apex_1-2	-4.47	27.85	-13.29	27.04	EVnA	0.02		0.90	25.23	0.38	
A	4.07	4.67 23.69 2.55 20.16 EVA EVnA 0.95	0.55		EVA	0.05		0.28	31.00	0.78	
Apex_2-3	4.67		0.95	0.95 0.34	0.27	22.77	0.79				
A	F 00		30.60 -11.79	28.24	EVA	0.00	0.54	1.62	31.00	0.12	
Apex_1-3	5.02 30	30.60			EVnA	0.39	0.54	1.59	24.27	0.13	

TABLE 4 Mean and Standard Deviation of the grey level values in immediate and delayed loading in stage B.

Variable	I.C.C.	F (0)	BE1	BE2	Stat. Sign
Comparison T1-T2	0,81	5,415	65	260	p<0.01
Comparison T2-T3	0,77	4,294	65	260	p<0.01
Comparison T1-T3	0,80	4,971	65	260	p<0.01

TABLE 5 Correlation between the 5 observers (Intra Class Correlation).

Levene's test for equality of variances, the researcher decides to adopt the appropriate F value that best corresponds to the data. This is to say that if Levene's test proves to be significant then one chooses the "EVnA" line otherwise the EVA line.

Stage B: The mean and standard deviation of the differences between the grey level values of subtraction images T1-T2Rec, T2-T3Rec and T1-T2Rec, at the ROIs (mesial, middle, apex) with immediate and delayed loading were assessed using t-test. They are summarized nTable 4.

No differences in grey values between immediate and delayed loading were observed in stage A as in stage B. (p<0.05).

Visual examination by observers

Table 5 summarizes the results of the visual evaluation of the five observers (raters) at T1-T2, T2-T3, and T1-T3 examinations. The intraclass correlation was used to access the reliability of raters. The values of this index range from 0 to 1 (Values approaching 1 indicate a strong correlation among the observers).

The Intra Class Correlation test is used when one wishes to test the consistency between data acquired from different observers concerning the same specimen (object), it presupposes scale level of measurement.

T=DF=Degrees of Freedom=represent the degree of variability of a measure.

No statistically significant variability at T1-T2, T2-T3 and T1-T3 was shown between the examinations (p<0.05).

Table 6 shows the mean and standard deviation of the visual comparison of the five observers related to immediate and delayed loading using t-test.

The visual evaluation of the radiographs, showed no stastistically significant difference between immediate and delayed loading. More specifically:

- a) examination T1-T2 (t 1.113, df 64, p>>0.05 NS); delayed (m -0.5, SD 0.49) and immediate loading (m -0.33, SD 0.71);
- b) examination T2-T3 (t 1.58, df 64, p>>0.05 NS);

	Delayed	Delayed		Immediate		Levene's Test					
	Mean	SD	Mean	SD		F	Sig.	t	df	Sig. (2-tailed)	
T1-T2 -0.5			-0,32			2 207	074	1 110	64	270	
	-0.5	-0.5 0.49		0,71	EVA	3,307	,074	-1,113	63,74	,270	
					EVnA			-1,176	7	,244	
			-0,4	0,56	EVA	5 700	010	1,496	64	,140	
T2-T3	-0,22	0,38				5,780	,019		63,78		
					EVnA			1,579	5	,119	
T1-T3	-0,65 0				EVA		100	9 -,900	64	,372	
		0,59	-0,5	0,72		2,641	,109		63,40		
					EVnA			-,929	5	,356	

TABLE 6 Mean and Standard Deviation of the visual examination of the five observers related to the loading factor.

delayed (m -0.22, SD 0.39) and immediate loading (m -0.4, SD 0.6);

c) examination T1-T3 (t -0.9, df 64, p>>0.05 NS); delayed (m -0.65, SD 0.58) and immediate loading (m -0.5, SD 0.72).

DISCUSSION

The results of the present study revealed no statistically significant variability in grey values between immediate and delayed loading at the cervical bone level in implants placed in the interforaminal area and restored with a telescoping overdenture. These results were confirmed both with the use of DSR and by visual evaluation of the radiographs. Given that grey values have been used as indicator of bone gain or loss in many studies (27-30) and their use in clinical trials was the only available option to evaluate and measure bone changes on radiographs, we consider our choice pertinent.

Although immediate loading of implants is "on the front burner" exhibiting an extreme clinical interest, studies focusing on bone changes in the cervical areas are limited. Assad et al. (2007) (31) based on the clinical examination and the periapical radiographs evaluation concluded that there were no differences in bone loss between immediate and delayed loading. Despite the difference in methodology between the aforementioned and the present study with respect to the subjected radiographic images the results are in accordance.

More recently, Geraets et al. (2012) (29) examined the bone loss along dental implants using digital subtraction on cropped PRs. They reported a significant decrease in grey value (0.6 units per month) around implants indicating a gradual bone loss on their mesial and/ or distal sides which is in discrepancy with our results probably because of the different methodology used. In detail, the time intervals between recalls (evaluated PRs) as well as the loading protocols of the present study differed. Geraerts et al. (2012) (29) did not use standardized time intervals and evaluated 3 different types of prosthetic restoration. In our study only cropped PRs obtained from patients following consecutive recalls at 0, 6 and 36 months were examined which represents a different procedure compared to Garaets' et al. (2012) (29). Additionally, our patients were restored with either an early or late protocol. Furthermore, the ROIs used in the study of Garaets' et al. (2012) (29) consisted of a "strip-like area", mesial and distal, along implants' surface. Also, each area-either mesial or distal-was measured as a whole. On the contrary, in the present study, 6 different ROIs (mesial neck, mesial middle, mesial apex, distal neck, distal middle, distal apex) were evaluated aiming to accurately determine the changes in bone level-as this is reflected by the differences in grey values-at the neck, middle and apex area. The authors believe that the discrepancy in the results between the two studies can be partly attributed to the different evaluation procedure of PRs.

Moreover, Carneiro et al. (2012) (28) also applied the method of DSR using standardized periapical radiographs that had been performed at specific time intervals (3, 6 and 12 months). Further, the estimation procedure of the gray level values of the subjected images was quite similar to that followed in the present study. Notably, in Carneiro et al. (28) study periapical radiographs were used, whilst, in the present study the radiographic images used were those of the single implants obtained from cropped PRs. Carneiro et al (2012) (28) concluded that the grey level values around implants that were loaded immediately increased during the first year of function. It is noteworthy, that the authors reported a significant decrease in grey value levels around implants which received conventional loading in a 12 months-follow up period. Additionally, no difference was observed among implants with different surface treatments. However, in

the present study no statistically significant variability in bone level was observed between immediate and delayed loading during the 36 months follow-up period either with the use of DSR or the visual assessment.

To the authors' knowledge, there are no published studies investigating the association between the visual assessment and DSR evaluation of bone around implants. In the present study, the visual comparison of the radiographic images, (T1-T2Rec, T2-T3Rec and T1-T3Rec) showed no statistically significant variability in the observations between immediate and delayed loading which was also supported by the DSR results. One may argue about the use of PRs in our study, but this radiographic assessment is widely used in clinical practice for the postoperative evaluation of the implants' sides (32). It is easy to obtain, allows evaluation of numerous implants' sides, is available in a digital form and also provides minimum exposure to radiation for the patient. On the other hand, PRs do not allow precise measurements as the accuracy of the depicted image is influenced by numerous factors. If standardized periapical radiographs instead of PRs had been used in our study, perhaps more accurate images might have been obtained. However, considering the location of the implants in the present clinical trial (anterior region of the mandible) it is doubtful whether the parallelizing technique could have been used, since the majority of the patients had shallow floor of the mouth.

Future investigations designed with a more standardized method to obtain PRs are needed in order to confirm the results of the present study. In addition it should be pointed out that, as stated in other studies, the evaluation of the grey value levels cannot be interpreted as a direct proof but rather as an indicator of bone change around implants.

CONCLUSIONS

Our study confirms that Emago[®] is a valuable method for bone level assessment around implants' neck even in digitized and cropped PRs. Furthermore grey value measurements in bone adjacent to either immediate or delayed loaded implants do not significantly differ after 3 years of function and visual assessment of the PRs images supports these findings.

Conflict of interest/ Disclosure statement

The authors declare no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Informed consent was obtained from all patients for being included in the study. This article does not contain any studies with human or animal subjects performed by the any of the authors.

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