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Quantification and Influence by IL-1β of the Pain and Inflammatory Response after Placement of a Cement-Screw-Retained Restoration

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Abstract: The objective of this study was to evaluate the pain and inflammatory response in soft tissues using healing and prosthetic abutments of different diameters and lengths. Methods: The study population was rehabilitated with Astra tech EV single implants (Dentsply Sirona, Atlantis, Dentsply Sirona S.A., Barcelona, Spain), 4.2 and 4.8 millimetres in diameter in upper and lower maxilla and loaded with custom abutments digitally designed with the software of the company Dentsply Sirona (Atlantis WebOrder, Dentsply Sirona S.A., Barcelona, Spain), version 4.6.5, with a larger diameter than the healing abutment, to account for biomarkers through ELISA. Results: Rehabilitations in mandible and with healing abutments below 4.29 millimetres and rehabilitators below 2.18 millimetres elicited higher pain and inflammatory responses, and in turn higher interleukin 1β values. Conclusions: Greater inflammation was evident in cases in which healing abutments with reduced diameter were used in relation to the same subsequent rehabilitation with prosthetic abutments of larger diameter.

Keywords: Screw-cemented retained implant prothesis; customised CAD-CAM abutments; peri-implant crevicular fluid; healing abutments; dental implants

1. Introduction

The influence of the formation of the supracrestal insertion tissue around implants is frequently studied because the peri-implant soft tissues are those that provide hygiene, aesthetics and health to a prosthesis or crown on implants, forming an aesthetic biological contour [1].

The dimensions of peri-implant soft tissues were described by Tomasi and collaborators based on the analysis of human biopsies [2]. Tomasi et al. in 2013 described soft tissue dimensions around 3.6mm while other authors claim an average of 4.20mm [3], including a barrier epithelium of 1.9mm and connective tissue of 1.7mm.

It was shown that the portion of the mucosa that is in intimate contact with the prosthetic abutment surface can be divided into two distinct zones: a marginal zone that harbours a junctional epithelium and a more apical zone that is composed of fibre-rich connective tissue [4–6].

These areas may vary depending on the cervical design, gingival biotype and implant depth.

From in vitro [7] and in vivo [8] experiments it was concluded that the junctional epithelium of the peri-implant mucosa through hemidesmosomes is adherent to the titanium surface, while other dog studies [4,9] suggested that the connective tissue in the interface zone has the character of a scar tissue (sparse in cells and vascular structures, but rich in collagen fibres), which is firmly attached to the abutment.

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Subsequent studies suggested that the vertical mucosal thickness necessary for establishing correct biological width around dental implants should be at least 2mm to avoid marginal bone loss [10,11]. It should be emphasised that the minimum and maximum thickness values serve as a starting point, when in fact what we are looking for is to give the patient back what they had, i.e. if a patient has a thin biotype where their gingiva measures 2.5 or 3mm, it is necessary to make the measurement and provide that gingival space at the time of acquiring a prosthetic abutment or when making the gingival contour of a provisional restoration.

It has been observed that it is possible to prevent peri-implant bone remodelling if we adapt the vertical position of the implant to the thickness of the soft tissue. Based on this principle, it is considered to submerge the implant from the gingival margin between 3 and 4mm depending on the size of the implant [12].

This study is significant because of the importance of biological aesthetic surround shaping in single-tooth restorations; the technique used will depend on the clinical approach of the case, the immediate or delayed placement of the implant and the need to improve the adjacent soft tissues. Studies by different authors showed the influence of inflammation, which could cause highly localised destruction of connective tissue and stimulate epithelial proliferation [13]. Predisposing factors are the shortage of space in the peri-implant tissues, use of inadequate attachments and poor patient hygiene, among others, which can lead to gingival recession, exposure of attachments and implant component; therefore, in response to inflammatory agents, infections or microbial endotoxins, a dramatic increase in IL-1β production by macrophages and other types of cells is observed [14]. IL-1β plays a central role in immune and inflammatory responses [15].

In particular, in this study we have evaluated the use of healing abutments with variable diameters and lengths to make the respective measurements that lead us to make appropriate decisions when performing second stage surgery and to use the attachment that favours the maintenance of hard and soft tissues in the long term.

An analysis of variables based on the diameter and height of the pillars was proposed. In this study, it was possible to observe the inflammatory and painful response in the maxillary and mandibular post-surgery sectors with single-unit rehabilitations on implants whose healing screw diameter was smaller than the diameter of the rehabilitating abutment.

The null hypothesis was that the greater the diameter of the abutment and the differences between diameters, the greater the pain and inflammatory response, with the consequent direct relationship of interleukin 1-β in these processes.

2. Results

A study with a total of 96 implants was planned for 96 patients, of whom 54 were women and 42 were men.

2.1. According to demographic data

2.1.1. Differences by gender

There were no statistically significant differences in the degree of pain perceived by the female sex, classified on average as 2.14 ± 1.37, not being very different from the degree of pain perceived by the male sex, classified as 1.90 ± 1.45.

However, with respect to inflammatory response by sex, greater differences were observed, since at 24 hours after placement of the rehabilitation the degree of inflammation in women was 0.33 ± 0.82 to 0.05 ± 0.22 in men, at 48 hours 0.44 ± 1.02 in women to 0.05 ± 0.22 in men, and on the day on which the inflammation subsided it was 1.89 ± 2.04 in women to 1.21 ± 0.98 in men.

IL-1β concentrations according to the degree of inflammation were 11.39 pg/ml for women and 7.12 pg/ml for men without statistically significant differences (Table 1).

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Table 1. Medians of pain and inflammation and IL-1B concentration with respect to sex at the time of placement (clinical).

Variable	Woman		Man	
	Average	D.E.	Average	D.E.
Degree of pain in clinic	2.14	1.37	1.90	1.45
Degree of inflammation in clinic	0.02	0.14	0.00	0.00
Concentration (pg/ml)	11.39	31.89	7.12	33

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. IL-1β concentration data were obtained by quantification using the ELISA technique. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at $p<0.05$. None of these results reached significance.

2.1.2. Differences by age

Regarding age, patients who were under 58 years old suffered less pain than those who were over 58 years old, specifically at 24 hours after rehabilitation placement; for those younger than 58 pain was classified by value of 0.24 ± 1.00, while for those older than 58 a value of 0.50 ± 0.96 was obtained, reaching a statistically significant difference ($p<0.05$).

Similarly, 48 hours after placement of the rehabilitation, for those under 58 a value of 0.16 ± 0.79 was obtained, while for those over 58 the value was 0.46 + 0.96, again reaching a statistically significant difference ($p<0.05$).

Consistently, those who were under 58 years old suffered less inflammation than those who were over 58 years old, specifically at 24 hours after rehabilitation placement; for those younger than 58 a value of 0.04 ± 0.20 was obtained, while for those older than 58 a value of 0.39 ± 0.88 was obtained, reaching statistically significant difference ($p<0.05$). Similarly, 48 hours after placement of the rehabilitation, for those under 58 a value of 0.04 ± 0.20 was obtained, while for those over 58 the value was 0.52 ± 1.09, again reaching a statistically significant difference ($p<0.05$). Finally, 72 hours after placement of the rehabilitation, for those under 58 a value of 0.06 ± 0.42 was obtained, while for those over 58 the value was 0.33 ± 0.87, also with a statistically significant difference ($p<0.05$) (Table 2)

Table 2. Differences in the degree of pain and inflammation with respect to the age of the patients.

Variable	Up to 58 years		Over 58 years		Sign.
	Half	S.D.	Half	S.D.	
Degree of pain in clinic	1.87	1.40	2.22	1.40	
Degree of pain in 24 hours	0.24	1.00	0.50	0.96	**<0.05**
Degree of pain in 48 hours	0.16	0.79	0.46	0.96	**<0.05**
Degree of pain in 72 hours	0.12	0.59	0.20	0.54	
Degree of pain until the day of pain remission	0.24	1.00	0.76	1.52	**<0.05**
Day when the inflammation subsides	1.36	1.24	2.09	2.01	**<0.05**
Degree of inflammation in clinic	0.00	0.00	0.02	0.15	
Degree of inflammation in 24 hours	0.04	0.20	0.39	0.88	**<0.05**
Degree of inflammation in 48 hours	0.04	0.20	0.52	1.09	**<0.05**
Degree of inflammation in 72 hours	0.06	0.42	0.33	0.87	**<0.05**
Degree of pain until the day of inflammation remission	0.14	0.76	0.85	1.79	**<0.05**
Day when the inflammation subsides	1.18	0.90	2.04	2.18	**<0.05**

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at $p<0.05$. None of these results reached significance.

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IL-1β concentrations according to the degree of inflammation were obtained for patients younger than 58 years of 3.99 ± 30.51 pg/ml, and 13.59 ± 33.54 pg/ml for those older than 58 years with statistically significant differences. ($p<0.05$) (Table 3).

Table 3. Medians of pain and inflammation and IL-1B concentration with respect to age at the time of placement (clinical).

Variable	Up to 58 years		Over 58 years		Significance
	Average	D.E.	Average	D.E.	
Degree of pain in clinic	1.87	1.40	2.22	1.40	
Degree of inflammation in clinic	0.00	0.00	0.02	0.15	
Concentration (pg/ml)	3.99	30.51	13.59	33.74	<0.05

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. IL-1β concentration data were obtained by quantification using the ELISA technique. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at $p<0.05$. None of these results reached significance.

2.1.3. Differences with respect to implant location

Regarding the location of the implants placed, those placed in the mandible presented a higher degree of pain than those placed in the maxilla. Specifically, statistically significant differences were observed 24 hours after placement of the rehabilitation since, in the maxilla, the degree of pain was assessed at 0.03 ± 0.18 while in the mandible it was 0.52 ± 1.16.

At 48 hours after placement, the difference was also statistically significant ($p<0.05$) with values of 0.00 ± 0.00 in the maxilla and 0.45 ± 1.05 in the mandible.

At 72 hours after placement, the difference was also statistically significant, since while the maxilla remained stable with values of 0.00 ± 0.00, those of the implants placed in the mandible were 0.23 ± 0.68 (Table 4).

Table 4. Differences in the degree of pain and inflammation with respect to the location of the implants.

Variable	Maxilla		Mandible		Sign.
	Half	S.D.	Half	S.D.	
Degree of pain in clinic	1.85	1.34	2.12	1.43	
Degree of pain in 24 hours	0.03	0.18	0.52	1.16	<0.05
Degree of pain in 48 hours	0.00	0.00	0.45	1.05	<0.05
Degree of pain in 72 hours	0.00	0.00	0.23	0.68	
Degree of pain until the day of pain remission	0.03	0.18	0.71	1.53	<0.05
Day when the inflammation subsides	1.13	0.72	1.98	1.93	<0.05
Degree of inflammation in clinic	0.03	0.18	0.00	0.00	
Degree of inflammation in 24 hours	0.00	0.00	0.31	0.77	<0.05
Degree of inflammation in 48 hours	0.00	0.00	0.40	0.95	<0.05
Degree of inflammation in 72 hours	0.00	0.00	0.28	0.82	quasi
Degree of pain until the day of inflammation remission	0.00	0.00	0.71	1.65	<0.05
Day when the pain subsides	1.00	0.00	1.88	2.00	<0.05

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at $p<0.05$. None of these results reached significance.

IL-1β concentrations according to the degree of inflammation were obtained, of 8.28 ± 33.77 pg/ml in maxilla, and 10.49 ± 29.88 pg/ml in mandible, without reaching statistically significant differences.

2.2. According to attachments used

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2.2.1. Healing cap or healing abutment

The pain and inflammatory response was evaluated based on the diameter of the healing abutment, which was always smaller in diameter than that of the rehabilitative abutment, in order to assess the soft-tissue response following placement of the rehabilitative abutment.

When the diameter of the healing abutment was less than 4.86mm, the degree of pain at the time of rehabilitative abutment placement was 2.46 ± 1.22, while when the diameter was greater than 4.86mm the degree of pain was 1.61 ± 1.45 ($p < 0.01$).

At 24 hours after rehabilitative abutment placement, the degree of pain when the healing abutment was less than 4.86 millimetres was 0.73 ± 1.30, while with a healing abutment greater than 4.86mm, the degree of pain was 0 ± 0 ($p<0.0001$).

At 48 hours after rehabilitative abutment placement, the degree of pain when the healing abutment was less than 4.86 millimetres was 0.60 ± 1.18, whereas with a healing abutment greater than 4.86, the degree of pain was still 0 ± 0 ($p < 0.001$).

At 72 hours after rehabilitative abutment placement, the degree of pain when the healing abutment was less than 4.86 millimetres was 0.31 ± 0.78, whereas with a healing abutment greater than 4.86, the degree continued at 0 ± 0 ($p < 0.01$).

Regarding the degree of inflammation, the results obtained are consistent with the data reported for pain. When the diameter of the healing abutment was less than 4.86 millimetres, the degree of inflammation 24 hours after placement of the rehabilitative abutment was 0.42 ± 0.87, whereas when the diameter was larger, the degree of inflammation was 0 ± 0 ($p < 0.001$). When the diameter of the healing abutment was less than 4.86 millimetres the degree of inflammation 48 hours after placement of the rehabilitative abutment was 0.54 ± 1.07, while when the diameter was larger the degree of inflammation was 0 ± 0 ($p < 0.001$). When the diameter of the healing abutment was less than 4.86 millimetres the degree of inflammation 72 hours after placement of the rehabilitative abutment was 0.38 ± 0.94, while when the diameter was larger the degree of inflammation was 0 ± 0 ($p<0.01$) (Table 5).

Table 5. Differences in pain and inflammation with respect to the diameter of the healing cap.

Variable	Up to 4.86mm		More than 4.86mm		Sign.
	Half	S.D.	Half	S.D.	
Degree of pain in clinic	2.46	1.22	1.61	1.45	<0.01
Degree of pain in 24 hours	0.73	1.30	0.00	0.00	<0.0001
Degree of pain in 48 hours	0.60	1.18	0.00	0.00	<0,001
Degree of pain in 72 hours	0.31	0.78	0.00	0.00	<0.01
Degree of pain until the day of pain remission	0.98	1.71	0.00	0.00	<0.0001
Day when the pain subsides	1.13	0.72	1.98	1.93	<0.0001
Degree of inflammation in clinic	0.02	0.14	0.00	0.00	
Degree of inflammation in 24 hours	0.42	0.87	0.00	0.00	<0,001
Degree of inflammation in 48 hours	0.54	1.07	0.00	0.00	<0,001
Degree of inflammation in 72 hours	0.38	0.94	0.00	0.00	<0.01
Degree of pain until the day of inflammation remission	0.96	1.86	0.00	0.00	<0,001
Day when the inflammation subsides	2.19	2.25	1.00	0.00	<0,001

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[1] Data on the degree of pain and inflammation were obtained by conducting surveys. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at p<0.05. None of these results reached significance.

IL-1β concentrations according to the degree of inflammation were obtained with abutments smaller than 4.86 millimetres of 13.81 ± 33.86 pg/ml, and 4.17 ± 30.51 pg/ml with abutments larger than 4.86 millimetres (p<0.01) (Table 6).

Table 6. Medians of pain and inflammation and IL-1B concentration with respect to scar plug diameter.

Variable on pain and inflammation according to healing abutment (diameter)	up to 4.86mm		over 4.86mm		Sign.
	Half	S.D.	Half	S.D.	
Degree of pain in clinic	2.46	1.22	1.61	1.45	<0.01
Degree of pain in 48 hours	0.60	1.18	0.00	0.00	<0,001
Degree of inflammation in clinic	0.02	0.14	0.00	0.00	
Degree of inflammation in 48 hours	0.54	1.07	0.00	0.00	<0,001
Concentration (pg/ml)	13.81	33.86	4.17	30.51	<0.01

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. IL-1β concentration data were obtained by quantification using the ELISA technique. The Mann-Whitney U has been applied since the variables under analysis do not have a normal dis-tribution. Statistical significance was established at p<0.05. None of these results reached significance.

2.2.1. Prosthetic abutment

Finally, the pain and inflammatory response was observed based on the abutment diameter of the prosthetic abutment, which was always larger than the diameter of the healing abutment used previously.

When rehabilitated with a prosthetic abutment diameter less than 5.86 millimetres, the highest degree of pain occurred at the time of placement, with a value of 1.69 ± 1.22, while with a prosthetic abutment greater than 5.86, the degree of pain was 2.39 ± 1.49. In this case, a statistically significant difference was observed (p<0.05). For the rest of the times evaluated, 24, 48 and 72 hours later, no statistically significant difference was observed.

The concentrations of IL-1β according to the degree of inflammation with prosthetic abutments smaller and larger than 5.86mm in diameter did not reach statistically significant differences.

On the other hand, with respect to the height of the prosthetic abutment, statistically significant differences were also observed when comparing the degree of inflammation observed with respect to the use of abutments larger and smaller than 2.45mm. At 24 hours after placement of the prosthetic abutment with a height less than 2.45 millimetres the degree of inflammation was 0.33 ± 0.78 while for an abutment greater than 2.45 millimetres it was 0.08 ± 0.45 (p<0.05). The same occurred 48 hours after placement of the prosthetic abutment with respect to the degree of inflammation, since for an abutment smaller than 2.45 millimetres the values were 0.46 ± 1.01 and for an abutment larger than 2.45 millimetres they were 0.08 ± 0.45 (p<0.05). Statistically significant differences were also observed at 72 hours, since for the abutment smaller than 2.45 millimetres the values were 0.33 ± 0.91 and with an abutment larger than 2.45 millimetres they were 0.04 ± 0.29 (p<0.05) (Table 7).

Table 7. Differences in pain and inflammation with respect to prosthetic abutment height.

Variable	Up to 2.45mm	More than 2.45mm	Sign.

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	Half	S.D.	Half	S.D.	
Degree of pain in clinic	1.99	1.35	2.08	1.46	
Degree of pain in 24 hours	0.48	1.09	0.25	0.86	
Degree of pain in 48 hours	0.46	1.01	0.15	0.71	**<0.05**
Degree of pain in 72 hours	0.21	0.62	0.10	0.52	
Degree of pain until the day of pain remission	0.69	1.50	0.29	1.03	
Day when the pain subsides	1.85	1.83	1.56	1.53	
Degree of inflammation in clinic	0.00	0.00	0.02	0.14	
Degree of inflammation in 24 hours	0.33	0.78	0.08	0.45	**<0.05**
Degree of inflammation in 48 hours	0.46	1.01	0.08	0.45	**<0.05**
Degree of inflammation in 72 hours	0.33	0.91	0.04	0.29	**<0.05**
Degree of pain until the day of inflammation remission	0.81	1.76	0.15	0.77	**<0.05**
Day when the inflammation subsides	1.98	2.10	1.21	1.03	**<0.05**

[1] Data on the degree of pain and inflammation were obtained by conducting surveys. The Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution. Statistical significance was established at $p<0.05$. None of these results reached significance.

The concentrations of IL-1β according to the degree of inflammation with prosthetic abutments less than 2.45 were 13.56 ± 34.72 pg/ml and with abutments greater than 2.45 were 4.42 ± 29.60 pg/ml, and they did not reach statistically significant differences.

3. Discussion

After analysis of the results, a greater inflammatory response was observed in the mandible than in the maxilla, both in the anterior and posterior sectors. Inflammation causes cell migration to areas where there is greater oxygenation, directly affecting the gingival margin of the restorations [16]. For this reason, it was decided to evaluate the existing inflammation margin and control it to avoid prolonged ischemia once the definitive prosthesis was installed.

The platform switching system also contributes to an improvement in inflammation control as it appears to help prevent peri-implant soft tissue recession over time compared to implants without platform switching [17,18], showing a positive effect on marginal bone levels compared to restorations without platform switching [19–21]. For this study, in all implants, the platform change concept was applied.

Regarding the attachments used, healing abutments with a diameter of less than 4.86mm showed greater pain and inflammation. IL-1β concentration reached levels of 13.81 ± 33.86 pg/ml for this diameter, and for a diameter greater than 4.86mm, it reached levels of 4.17 ± 30.51 pg/ml, with statistically significant differences between these diameters at $p<0.01$.

On this point, there are studies that reveal benefits of modifying the healing abutments in immediate surgeries by promoting the formation of stable and thicker peri-implant tissues [22]. Gamborena et al. (2015) asserts that using abutments of a smaller custom diameter can provide the following advantages: support for connective tissue grafts in the most coronal position, improved papilla formation, allowing primary flap closure, providing support for an immediate provisional restoration or a restoration bonded to adjacent teeth, as is the case with Maryland bridges, and eliminating vertical loading of the grafted tissue during the healing phase [22]. In the long term, the use of this narrower customised abutment allows the thickness of the tissues initially operated on during the first surgery to be maintained [23].

When direct-to-implant prosthetic abutments are used, the probability of cervical to apical migration due to continuous gingival inflammation, in addition to the number of times the attachments are connected and disconnected, generating repeated changes, which does not occur with the use of a transepithelial abutment that avoids this constant manipulation of soft tissues

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[24,25], in addition to reducing the micro-space that may exist at the implant-abutment interface [24]. However, the use of conventional abutments showed a clinically acceptable microgap as reported in the scientific literature [26–29].

It should be clarified that transepithelial abutments were not used for this study, but that all abutments were exactly the same to avoid the risk of bias for this reason. Significant changes in pain were observed with the use of prosthetic abutments with platforms larger than 5.86mm, thus, the larger the diameter of the prosthetic abutment, the greater the pain response, reaching levels of significance only on the day of prosthetic rehabilitation placement ($p<0.05$). Both the prosthetic abutments and the definitive crowns were designed without altering the same work protocol, in order to obtain the same result in all cases, even in those where there may have been implants with angulations that could require corrections. Although there are studies indicating that the process of overlaying the titanium custom abutment with the pre-scan custom abutment library data improved the accuracy of a digital scan performed with respect to an intraoral scan, there was no risk of bias because all cases were scanned with the same scanning abutments [30,31]. Studies have shown that the use of customised abutments leads to an increase in the final abutment size, improved retention of the prosthetic work and reduced angulation of the abutment in relation to the implant axis, thus reducing the risk of unscrewing or fracturing the dental screw, and that the use of customised abutments provides stability to aesthetically compromised peri-implant tissues [32,33].

The final crowns were milled in the dental laboratory with multilayer zirconium oxide discs, complying with the cementing protocol for a cement-screw system, using dual polymerisation self-adhesive cement in an indirect way, highlighting the importance of this technique involving the elimination of cement remains that can cause constant inflammation in the gums, to be finally installed in each of the 96 patients. There are studies that indicate that the cemented option is reliable, but others indicate that cemented restorations were associated with a higher rate of biological complications with respect to screw-retained restorations [34–36].

Studies on the level of IL-1B in crevicular fluid comparing cemented and screw-retained implant-supported rehabilitations concluded that the clinical and radiographic parameters showed no difference in the volume of peri-implant sulcular fluid, and thus were comparable to each other in terms of clinical-radiographic status with IL-1β levels within the normal range [37]. In this study all the restorations were screwed to implants so that there was no risk of bias in this aspect. Under similar conditions, the level of IL-1β was much higher in peri-implant crevicular fluid than in gingival crevicular fluid [38], so it is expected that in implant-supported rehabilitations IL-1β values will be higher. Studies indicated that painful and inflammatory processes were associated with increased levels of IL-1β. Higher levels of proinflammatory cytokines (interleukin (IL)-1β, IL-6) were observed in individuals with peri-implantitis compared to healthy implants [39–41]. In this study, similarly, IL-1β levels were higher the greater the pain and inflammatory response, especially when the healing abutment was less than 4.86 millimetres with a pain grade of 0.73 ± 1.30, whereas with a healing abutment greater than 4.86, the pain grade was 0 ± 0. The statistically significant difference was $p<0.0001$. Similarly, statistically significant differences with a p-value <0.001 were shown in the degree of inflammation with the comparison between the use of healing abutments smaller and larger than 4.86 millimetres.

4. Materials and Methods

4.1. Type of study

Prospective observational cohort study approved by the Andalusian Biomedical Research Ethics Coordinating Committee (Code US-DTL-2022.1) that complies with all the guidelines of the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [42].

This is an observational study whose invasive procedure was the collection of saliva samples from the peri-implant tissues of implants rehabilitated with different diameters of healing and prosthetic abutments.

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All patients signed an informed consent form based on this study and understood and accepted the type of treatment carried out on them.

4.2. Patient selection

A series of patients meeting the following inclusion and exclusion criteria were selected:

Inclusion criteria:

- Adult patients over 40 years of age
- Edentulous patients requiring single crowns
- Single edentulous spaces 6 to 8mm in height

Exclusion criteria:

- Patients with uncontrolled chronic diseases
- Patients with immune diseases
- Patients with smoking habits, alcoholism or narcotic drug use
- Patients medicated with steroids or bisphosphonates
- Patients with active periodontal disease
- Patients with poor hygiene habits

4.3. Surgical procedure

Patients were surgically treated with implants (Astra tech EV, Dentsply Sirona S.A., Barcelona, Spain) of 4.2mm or 4.8mm diameter in the upper and lower jaw.

The implants received healing abutments and were subsequently connected with customised abutments (Atlantis, Dentsply Sirona S.A., Barcelona, Spain), which were digitally designed with the software (Atlantis WebOrder, Dentsply Sirona S.A., Barcelona, Spain), (Atlantis Design, (VAD) version 4.6.5, which provided the necessary precision, the combination of biological, anatomical and engineering parameters providing the beneficial conditions for the soft tissue healing and adaptation of the final restoration.

All prosthetic abutments were individualised with a larger diameter than the healing abutment in order to evaluate the pain and inflammatory response of patients after definitive rehabilitation, which was quantified by the presence of biomarkers present in the crevicular fluid such as interleukin 1 beta (IL-1β) proteins in an ELISA test.

4.4. Prosthetic procedure

The material of choice for the preparation of customised abutments was Zirconium Oxide (InCoris ZI meso, Dentsply Sirona, DeguDent GmbH Rodenbacher Chaussee Hanau-Wolfgang, Germany) for CAD/CAM production, the same abutment design was used for all patients, the only variant was the measurement of the available soft tissues and according to that the surface to make the contact with the critical and subcritical profile, what we now know as B,C,E zone [43].

For the prosthetic crown, zirconium oxide discs were chosen (Cercon XT ML, Dentsply Sirona, DeguDent GmbH Rodenbacher Chaussee Hanau-Wolfgang, Germany) which has 750 MPa of resistance to bending over its entire length, taking the precaution of maintaining a minimum thickness of the restoration of 1.5mm as a margin of error and the advantage of having a 49% translucency in degradation throughout the restoration, obtaining a more aesthetic result.

The cases were sent to the dental laboratory, which continued with the milling process of the single-unit restorations on implants.

The cases were planned to be of the indirect cement-screwing type. Dual-curing self-adhesive resin cement (Relyx unicem 2, 3M ESPE AG, Dental Products Seefeld, Germany) was used because of its dimensional stability and high resistance to microfiltration due to long-term dissolution of the material.

Finally, the single crowns were installed in each of the patients and the pain scale was performed immediately.

4.5. Pain and inflammation scales

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These were manually annotated on paper with a template for each patient, which consisted of variables from 0 to 5, for greater patient comprehension, with 0 being no pain and 5 being maximum pain. Specifically, value 0 was assigned to those patients who had no pain at any time during the placement or afterwards, value 1 was assigned to those patients whose pain did not last more than 10 minutes, value 2 to those patients whose pain disappeared before 24 hours, value 3 to those whose pain disappeared between 24 and 48 hours after placement, value 4 to those whose pain disappeared after 72 hours, and value 5 to those whose pain lasted for more than 72 hours.

The inflammation scale was also evaluated, also consisting of variables from 0 to 5, and scored manually on paper with a template for each patient, being 0 no inflammation and 5 maximum inflammation. Specifically, value 0 was assigned to those patients who had no inflammation at any time during or after placement, value 1 was assigned to those patients whose inflammation did not last more than 10 minutes, value 2 to those patients whose inflammation disappeared within 24 hours, value 3 to those whose inflammation disappeared between 24 and 48 hours after placement, value 4 to those whose inflammation disappeared within 72 hours, and value 5 to those whose inflammation lasted for more than 72 hours.

4.6. Sampling

Sample collection was carried out 4 hours after delivery of the rehabilitation to each patient.

The collection of inflammation biomarker samples was taken at 4 sites (mesiobuccal, distobuccal, mesiolingual and distolingual) of each implant, avoiding contaminating the sample with saliva, a sterile paper collection strip (PerioPaper strips, Oralflow, Smithtown, NY) being inserted into the peri-implant sulcus for 30 seconds according to the manufacturer's instructions.

The four strips from each implant were pooled in Eppendorf centrifuge tubes and subsequently stored at -80°C until further processing.

4.7. Biomarker analysis using ELISA technique for IL-1β

The kit used for the ELISA technique was the KIT Quantikine® HS ELISA Human IL-1β/IL-1F2 Immunoassay (Ref. HSLB00D). It is a sandwich-type enzyme immunoassay technique in which the plate is treated with a monoclonal antibody specific for human IL-1β, so that in the presence of IL-1β in the sample, the antibody fixed on it will bind. the plate. On the day of processing, they were thawed and 400µl of Calibrator Diluent RD5T were added, the tubes were homogenized by vortexing so that they came into contact with the solution and centrifuged 3 times at 15,600G for 5 minutes at 4ºC. This elution was divided into 4 aliquots of 100µl, only one being used for the test.

Before ELISA, the samples are diluted by mixing 10µl of sample in 390µl of RD5T buffer (0.025) as indicated in the protocol, thus obtaining a 40x dilution. This step was repeated twice and left at 80x.

In total, 40 samples (in duplicate) and 8 standards (in duplicate) were used, making a total of 96 wells.

The procedure begins by adding 50µl of the RD1-63 test diluent to each well. After this, 100ul of standard or sample are added per well and covered with an opaque adhesive sticker for incubation for 2 hours at room temperature in a plate shaker at 500rpm. Afterwards, it is turned upside down and dried face down on paper. The process is repeated 3 times for 4 washes. Each wash is carried out with 400µl of Wash Buffer (reference 895003 of the kit itself, Ref. HSLB00D). After the last wash, all of the Wash Buffer is removed by inverting the plate and drying on clean paper.

Once this is done, 200µl of Human IL-1β HS conjugate is added to each well and covered again with a new adhesive sticker for incubation for 1 hour at room temperature while shaking. Subsequently, a new wash is carried out. After that, 200µl of Streptavidin Polymer-HRP (1X) is added to each well and it is covered again with a new adhesive sticker for incubation for 30 minutes at room temperature on the plate shaker at 500rpm. The washing is repeated and 200µl of Substrate Solution is added (100µl of color A + 100µl of color B) and incubated for 30 minutes at room temperature on the bench, well protected from light.

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Finally, 50µl of Stop Solution is added to each well and resuspended. The colour of the wells then changes from blue to yellow.

The results are read before 30min with a λ= 450 nm. To correct the absorbance, another reading of λ= 570 nm is taken, due to possible imperfections in the plate. The reading was carried out on the Thermofisher MultiScan Go spectrophotometer.

4.8. Interpretation of samples

The protocol that was followed was to take the average of the two readings of each sample or standard. To obtain more precise results, the average was subtracted from the absorbance that gave zero.

A corresponding working standard curve was created according to the absorbances obtained in the standards, representing the absorbances on the Y axis and the known concentration of the standards on the X axis. The X was removed from the equation of the straight line and the calculation was carried out for each absorbance of the samples obtained.

Straight line: y= 0.1892x + 0.0295

If the samples had been diluted, the measured concentrations were multiplied by the dilution factor.

The minimum detectable dose was 0.033pg/ml.

4.9. Statistical Analysis

The Kolmogorov-Smirnov test has been applied to determine the normality of the numerical variables, concluding that, for the variables under analysis, in no case is the distribution normal.

To cross-check qualitative variables, the Chi^2 test was carried out. To determine the groups that make the difference, Haberman's corrected standardized residuals have been used, which has made it possible to obtain the significance of the cells independently. This significance implies that the percentage of the cell is different, statistically, from that corresponding to the total. of the sample.

For the processing of categorical and numerical variables, the Mann-Whitney U has been applied since the variables under analysis do not have a normal distribution.

Given that the target variables follow a non-normal distribution, Sperman's correlation has generally been applied.

Statistical significance has been indicated with the usual format ($p<0.05$; $p<0.01$; $p<0.001$, $p<0.0001$ and $p<0.00001$), the lower the figure the greater the significance.

5. Conclusions

Greater inflammation was evident in cases in which reduced diameter healing abutments, larger diameter prosthetic abutments and lower height abutments were used. The greater the degree of pain and inflammation, the higher the level of IL-1β. The length of the healing abutments must be longer than the initial gingival measurement to avoid future inflammations.

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