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# **Pain Evaluation Between Stainless Steel and Nickel Titanium Arches in Orthodontic Treatment – A Comparative Study**

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Additional information is available at the end of the chapter

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## **1. Introduction**

The pain has traditionally been one of the most common side effects in orthodontic treatment. Orthodontic movement causes an inflammatory reaction in the periodontium and the pulp, which stimulates the production of biochemical mediators that cause the sensation of pain [1].

Different factors such as gender, personality and previous experience with other dental treatments may influence the concrete experience that each patient experiences with a particular orthodontic treatment [2].

It has been described by several authors [3-10] that pain begins at 4 hours after application of the force, after 24 hours, it descends, maintaining a plateau of lower intensity for two or three days, to continue descending from the fifth and sixth day until it disappears.

In the beginning, the pain was evaluated in a subjective manner, though in recent decades, numerous studies [11-12] have focused on the composition of crevicular fluid and changes that occur in it during orthodontic treatment as a more objective assessment of pain.

The application of mechanical forces moves the tooth and induces an inflammatory reaction by compression of the periodontal ligament. As a result, a variety of mediators are produced within the periodontal space, spread out at the crevicular fluid and reflecting the biological processes taking place. Several *in vivo* studies have used crevicular fluid analysis to monitor changes.

Crevicular fluid analysis is a noninvasive study of the cellular responses of the periodontal ligament during orthodontic treatment [13]. There are a variety of substances involved in the bone remodeling, produced in the cells of the periodontal ligament, that are spread out in the crevicular fluid [14].

Three substances, interleukin 1 $\beta$  (IL-1 $\beta$ ), prostaglandin E2 (PG-E2) and substance P (SP) were independently associated with pain [15-17], and are expressed during initial tooth movement in sufficient amounts to be detected in the crevicular fluid [18].

## 2. Problem statement

95% patients perceive pain during orthodontic treatment [6, 19], this pain being an important factor in rejecting treatment [20] or in interrupting it [21]. The pain involved has been described by various authors [3-10], there being different factors that modify it; gender, personality and previous experience with other dental treatments [2].

Ogura *et al.* [22] found a relationship between the magnitude of the force applied on the tooth and pain response, although other authors did not [23-25]. Additionally, the type of force (continuous or non-continuous) is also important. High and non-continuous forces [11,26,27] tend to significantly reduce the levels of IL1 $\beta$  at 168 hours from applying the force, which suggests the need for reactivation in order to maintain a sufficient production of IL1 $\beta$ . These types of forces not only increase the risk of radicular resorption on raising the hyalinization of the periodontal tissue [28,29], but also induce very sharp peaks of rises and falls in cytokine levels of which lead to undesirable results on the tissular level and the need for reactivating the forces. Light and continuous forces, however, tend to maintain high levels of IL1 $\beta$  so the need for reactivation is diminished [30-32]. These forces keep cytokine levels, which are necessary for continuous periodontal remodelling, high for a longer time.

The efficiency of orthodontic forces with different intensity and different duration has long been a major problem in the orthodontic clinic. In this study, to evaluate the efficacy and duration of each type of orthodontic force inducing initial tissue reaction two potent mediators of pain and bone resorption were measured; Prostaglandin E2 (PGE2) and substance P (SP).

Lastly, the material of the archwires that are fitted in the mouths of patients stainless-steel (SS) and nickel-titanium (Ni-Ti) exercise the force that may have an influence on pain, although there is controversy on this point [33]. There are few studies that compare pain depending on the type of archwire employed.

The aims of this work were, therefore:

- To compare pain during the initial stages of orthodontic treatment depending on the type of archwire employed; stainless steel (SS) or nickel-titanium (Ni-Ti).
- To determine a mathematical equation for predicting the level of pain depending on the time elapsed from fitting the archwire and that, therefore, would allow us to obtain the

moment of peak of pain and to establish the moment when pain begins and ends depending on the type of archwire.

- To determine the difference in pain between time intervals.
- To analyse the crevicular fluid samples taken from patients to whom it has been performed the subjective study of pain.

### **3. Application area**

A comparative, prospective clinical study was carried out at the Orthodontics Teaching Unit of the University of Valencia, Spain from January to April 2010. The study had previously been approved by the Ethics Committee of the University of Valencia. Rights have been protected by an appropriate Institutional Review Board and written informed consent was granted from all subjects. The Helsinki declaration was considered and its guidelines were followed in our investigation. All patients agreed to participate in the study, even though the diagnosis material was gathered as part of their treatment protocol.

## **4. Material and methods**

### **4.1. Sample**

A total of 150 patients who presented themselves at the Master in Orthodontics in order to receive Orthodontic treatment were selected.

The following inclusion criteria were established:

Patients who were to undergo a fitted Orthodontic treatment without dental extractions.

The presence of bracket cementing throughout the upper and/or lower arch.

The presence of good oral and periodontal health.

Whereas the exclusion criteria were:

The taking of any drug during the study.

The presence of active two band dental appliances during the treatment that would cause additional pain.

The presence of extra-oral appliances during the treatment that would cause additional pain.

On applying all these criteria, we obtained a total of 112 patients with a mean age of 19.8 years, ranging from between 9.5 and 64 years old. The sample comprised 37 males and 75 females.

The sample was divided according to the type of archwire that each of the patients wear: 49 patients with stainless-steel (SS) archwires and 63 patients with nickel-titanium (Ni-Ti)

archwires. Of the 49 patients with SS archwires, 31 were females and 18 males and of the 63 patients with Ni-Ti archwires, 44 were females and 19 males.

Table 1 shows the distribution of the sample depending on age, gender, archwire type and according to where their archwires were fitted (upper, lower or both arches).

Archwire type	Female	Male	Arch upper	Arch lower	Both arches	Age (mean)
Ni-Ti Archwire (N=63)	44	19	38	25	4	22.6
Stainless-Steel Archwire (N=49)	31	18	31	18	2	17.2

**Table 1.** Sample distribution according to gender, the arch on which the archwire was fitted, age and archwire type used.

#### 4.2. Method

After completing the appropriate orthodontic diagnosis, bracket bonding, which was carried out by 8 previously trained students of the Master of Orthodontics, Faculty of Medicine and Dentistry, University of Valencia, was scheduled.

Once the bonding of the brackets was performed, a 0.12" (diameter) Ni-Ti or SS arches were placed randomly in the patients enrolled in the study in order to compare the difference between groups in relationship with pain. In order to standardize the protocol, o elastomeric ligatures were used in all patients to hold the arches into the bracket system. The placement of the selected type of archwires did not alter the treatment of each patient.

### 5. Subjective assessment of pain — Patient questionnaire

First of all, a questionnaire was designed in order to assess the subjective level of pain. Having fitted the orthodontic appliance and the different archwires (SS and Ni-Ti), the patients filled in a pain questionnaire especially designed for this study, specifying the amount of pain (0=No pain; 1=discomfort; 2=slight pain; 3=intense pain) they experienced each day (from day 1 to day 14) and the time of day (morning, afternoon and night) they felt it. They were instructed to stop filling in this questionnaire after two consecutive days with an absence of pain. By doing so, the subjective values were obtained on an arbitrary scale. The appraisal of the questionnaire allowed us to assess both the subjective level of pain at each moment after the archwire was fitted and the total pain level experienced during the entire process of adapting to the archwire, obtained as the sum of the reported pain.

## 6. Objective assessment of pain — Crevicular fluid analysis

Secondly, an objective evaluation of pain was performed by analyzing the biochemical pain mediators in the crevicular fluid, in the laboratory.

The crevicular fluid samples were taken at the following stages of orthodontic treatment:

Before bracket bonding.

After 24 hours of placement of the archwire (Stainless Steel or Nickel Titanium).

A week after the archwire and bracket placement.

One month after the positioning of the initial archwire.

Crevicular fluid, using sterile paper strips (Periopaper Strip®. Proflow Incorporated. New York), was collected. Each sample was collected according to the technique described by Offenbacher et al. [34] and later modified by Uematsu et al. [11], without removing the plaque of the tooth in order to not alter the outcome of the study. However, efforts were made to collect the sample in the absence of plaque. The collection of all the crevicular fluid samples was taken by the same observer.

The technique used for sample collection was made by: firstly, drying of the mouth with suction; after that, isolating the area with cotton rolls;thirdly, looking for drying places where the paper strip is placed; and then, taking the sample of crevicular fluid by placing the Periopaper® in the binding groove between the tooth and gum. The paper strip is kept in this position within 30 seconds; and finally, the samples are placed between the sensors of the Periotron® 8000 (Proflow Incorporated. New York. USA) in order to obtain the crevicular fluid collection in Periotron units (Figures 1 and 2).

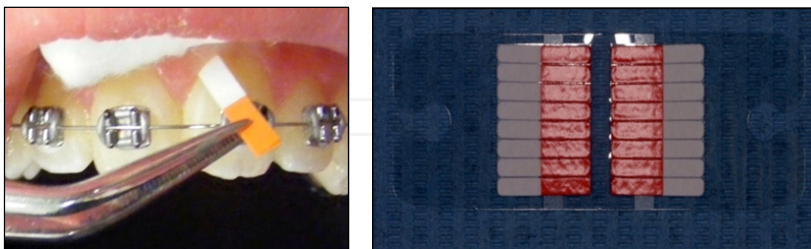


Figure 1. Sample collection of crevicular fluid with Periopaper®.

The extracted samples obtained were measured by ELISA immunofluorescence technique. To quantify the levels of substance P and PGE2, all samples were measured in duplicate. In our case, kits of high purity of the R & D Systems (Inc Minneapolis brand, USA) were used.

The spectrophotometer UV.vis shown in Figure 3 was used for the sample analysis. The spectrometer is an instrument used in biochemical analysis that measures, as a function of

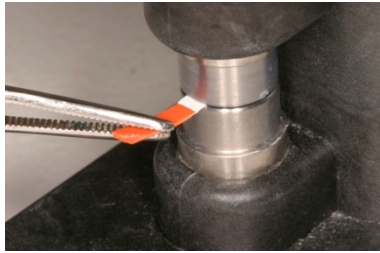


Figure 2. Crevicular fluid sample placement between Periotron® sensors.

wavelength, the relationship between the values of the same photometric magnitude related to two beams of radiation and the concentration and chemical reactions that are measured in a sample.



Figure 3. Spectrophotometer UV. vis

The Periotron® (Figure 4), must be properly calibrated before its use. It consists in a device for measuring the volume of the gingival crevicular fluid, collected by the paper strips (Periopaper®). Usually, a number, defined as Periotron unit, appears on the screen; constructing calibration graphs is required to obtain microliters, by using known amounts of fluid.

Once the device is switched on, it should be heated for 10 minutes. Then, it should be set to zero. After that, a dry paper strip must to be placed inside and the dial has to be adjusted until the zero value appears on the digital screen. A Hamilton microsyringe is then used (maximum volume 2µl, with 0.02µl gradations) to dispense known volumes of calibration liquid (human serum, being similar to the gingival crevicular fluid viscosity and composition) in the paper strips.

In our study, the paper strips were transferred to the Periotron® sensors (2-3 seconds) quickly to avoid evaporation errors. These paper strips were positioned in a standardized way, with the orange tips off the sensors.



Figure 4. Periotron® machine.

After about 16 seconds, the Periotron Unit of each of the samples analysed was obtained. This occurred when the screen went from position I to position II in the frontal side of the device. Using moistened gauze with alcohol, the Periotron® sensors were cleaned between each sample.

Each volume was measured at least three times and the machine was set after each sample to zero.

In this way two variables were obtained:

1. The volume (in  $\mu\text{l}$ ) of serum dispensed with Hamilton microsyringe (Figure 5).



Figure 5. Hamilton Microsyringe.

2. The Periotron Unit values of each of the parameters of the sample (average of the measurements made three times).

With these measurements, a linear regression curve was performed, obtaining a formula of the type  $y=ax+b$ , where "a" is the slope of the curve, "b" the intersection of the axis, and "x" in the crevicular fluid in Periotron Units.



## 6.1. Statistical method

The normality of the total pain (TP) distribution experienced by the patients throughout the treatment was checked using the Kolmogorov-Smirnov test, which allows us to compare the means of independent samples using Student's *t*-test.

A two-factor (time and archwire type) ANOVA was applied with the Scheffé test for multiple comparisons.

The  $\chi^2$  test was used to analyse factor dependence.

Non-linear regression was used for the variables, PL: pain level and T: time elapsed from fitting the dental archwire, with an estimation of best-fit parameters and quality assessment of the same through  $R^2$ , which indicates the percentage of variation of one variable that can be explained by the variation of the other.

## 7. Results

The total pain (TP) experienced throughout treatment, the pain level (PL) associated with each time section (morning, afternoon, night) and, therefore, the time elapsed from the beginning of treatment and the peak of maximum pain experienced have all been analysed.

### 7.1. Subjective study of pain

#### 7.1.1. Total pain associated with treatment

From the data provided by the patients in the questionnaire, the total subjective pain (TP) reported by each patient throughout the study was determined as the sum of pain level at each time of the day.

With both types of archwires, there was no case of pain after the tenth day onwards so, in the study that follows, we are only going to consider the data up until that moment, which amounts to 232 hours following the fitting of the initial archwires. With these considerations, the maximum TP possible would be 90 points, even though the maximum value suffered was 48 points for the SS archwires and 36 points for Ni-Ti archwires.

TP data distribution for each type of archwire (Ni-Ti and SS) corresponded to a normal distribution with  $p > 0.850$  and  $p > 0.150$ , respectively. Data on level, mean, standard deviation and percentiles of 25% and 75% for each type of archwire and for the total of the sample are presented in Table 2.

It could then be confirmed that the mean pain value for SS archwires was greater than for Ni-Ti archwires with  $p < 0.007$ .

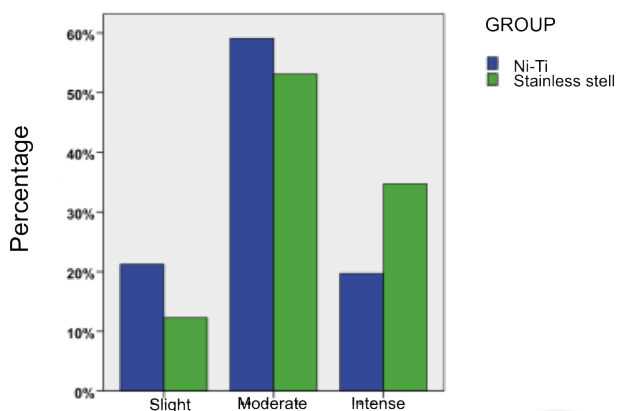
Using the percentile values of the total group, the pain suffered throughout the treatment can be classified as: "slight" for under 10 points; "moderate" for between 10 to 20 points; and



Treatment	mean	SD	Minimum	Maximum	Percentile 25%	Percentile 75%
Ni-Ti	14.6	7.2	0	36	10	19
SS	19.8	11.5	3	48	11	29
TOTAL	16.8	9.5	0	48	10	21

**Table 2.** Total pain (TP) over the 10 days following the beginning of treatment with Ni-Ti or SS archwires regardless of the archwire used. Mean and standard deviation (SD), minimum and maximum values and the percentiles of 25 and 75% were also presented.

“intense” for over 21 points. Applying this criterion, figure 6 shows the percentage of TP suffered using the two types of archwire in the study.



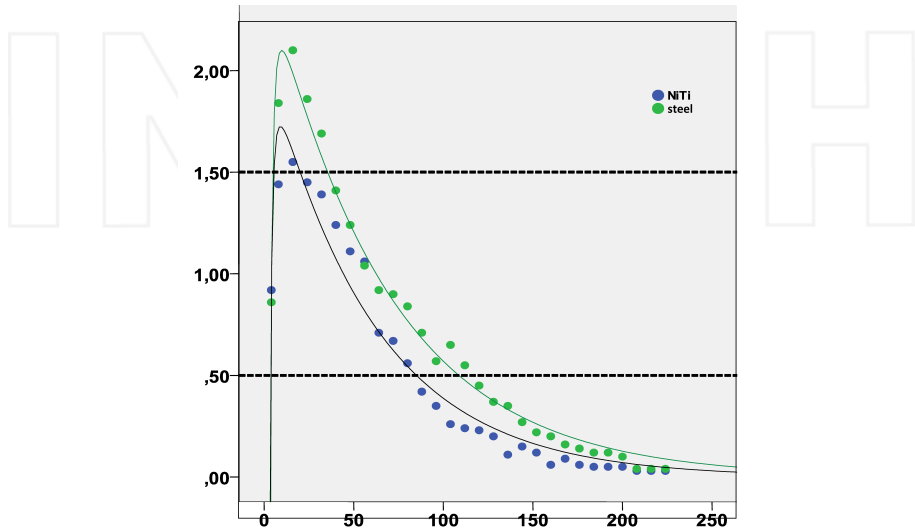
**Figure 6.** Percentage of total pain, slight (less than 10 points), moderate (between 10 and 20 points) and intense (over 20 points) for the two types of archwire studied (SS and Ni-Ti).

Dependence between the pain level and type of archwire was found to be  $p < 0.006$ . It can be observed that the percentage of cases with intense pain in the SS group, 34.7%, is greater than in that of the Ni-Ti group, 19.7%, whereas in the case of slight pain, the percentage is the other way around being greater in that of the Ni-Ti group with 21.2% than the 12.2% of the SS group, although, in this case, without statistically significant difference.

Furthermore, the mean of the days that the patients experienced pain was 4.84 days in general, with 4.5 of mean for patients with Ni-Ti archwires as opposed to 5.4 days of mean for the group with SS archwires, with a statistically significant difference of  $p < 0.04$ .

### 7.1.2. Pain level depending on the time elapsed from beginning of treatment

The subjective pain level (PL) for each time period was analysed according to the pain experienced during those same periods. As has been indicated in Material and Methods section, the possible evaluation in this case ranges from 0-3 points. The experimental results for each type of archwire are shown in figure 7.



**Figure 7.** Experimental results and fit to the proposed curve  $PL = a \cdot T^{-3} + c \cdot \exp(-b \cdot T)$  for the two types of archwires studied. PL values have been categorized as slight: less than 0.5; moderate from 0.5 to 1.5; and high: greater than 1.5.

It can be observed that, in general, the shape of the curve is similar for both types of archwires, pain appearing after a few hours and decreasing gradually. This similarity in pain behaviour encouraged us to look for a mathematical function that would fit the points of the curve in order to evaluate pain depending on time and that, therefore, would provide a predictive study of the pain associated with the archwire or with the physiological characteristics of the process them self.

Given that the representation of pain level (PL) with the time period, regardless of the archwire used, also had a similar shape to that shown in figure 7, we then analysed this situation, as here the number of cases was higher and the fitting of the mathematical function would have more statistical validity and offer information on the evolution of pain in general. The points were fitted using non-linear regression curves of different types, the maximum fit being found to be a curve of the form:  $PL = a \cdot T^{-3} + c \cdot \exp(-b \cdot T)$ , where PL, is pain level, T is the time elapsed in hours and the values "a", "b" and "c" correspond to the best-fit parameters that could differ or not depending on the type of archwire. The curve chosen for fitting consists of two terms: a decreasing exponential in which the parameters "b" and "c" are related, respectively, to the

fall in the exponential and to PL in each time period, and a potential term that becomes insignificant for long times, but which modulates the growth of the exponential for low times, being responsible for the height that the peak of the curve reaches and is characterized by the “a” parameter. In this way, the first term of the equation and, therefore, the “a” coefficient is important in the first hours following the fitting of the archwire and represents the higher or lower PL corresponding to the “peak”. The “b” parameter indicates the greater or lesser speed in the reduction of pain and value “c” represents the higher or lower PL throughout the treatment.

The parameters of fit for the 3 cases considered (pain in general regardless of the type of archwire, pain due to Ni-Ti archwire and pain due to SS archwire) are shown in Table 3. The goodness of fit for the three cases analysed is provided by the correlation coefficient R<sup>2</sup>, which is very high, 0.983, 0.964 and 0.987, for the total group, the SS archwires group and the Ni-Ti group respectively.

	Parameter	Estimation	Typical error	IC 95%		R <sup>2</sup> value
				Lower limit	Upper limit	
Total	a	-83.1	6.4	-96.2	-70.0	0.983
	b	0.016	0.001	0.015	0.017	
	c	2.31	0.07	2.17	2.45	
SS archwires	a	-70.4	8.8	-88.5	-52.4	0.964
	b	0.017	0.001	0.015	0.019	
	c	2.12	0.09	1.93	2.32	
Ni-Ti archwires	a	-100.8	6.1	-113.3	-88.2	0.987
	b	0.015	4.8 E-4	0.014	0.016	
	c	2.56	0.06	2.43	2.68	

**Table 3.** Values of parameters (a,b,c) and IC95% of the same corresponding to the non-linear fit of pain level (ND) depending on time in hours (T) elapsed since the beginning of treatment for the two types of archwires used and for the set of all cases. The non-linear fit corresponds to the expression  $PL=a * T^{-3}+c * \exp (-b * T)$

The values of the best-fit parameters indicate that: patients with SS archwires have a higher pain level at first than Ni-Ti patients (statistically significant difference in parameter a); a parallel reduction in pain level takes place (statistical equality in parameter b); but during the entire treatment patients with SS archwires experience more pain (statistically significant difference in parameter c).

Figure 7 also shows that after the tenth day no patient reported any pain, which is why our study ended at that point, 232 hours from the fitting of the archwire. The moment of the pain disappearing was practically the same for the two types of archwires, but it is interesting to calculate the average time that pain may be considered as high, moderate or slight. To do so, the PL experienced during the treatment was categorized on these three levels. The criterion

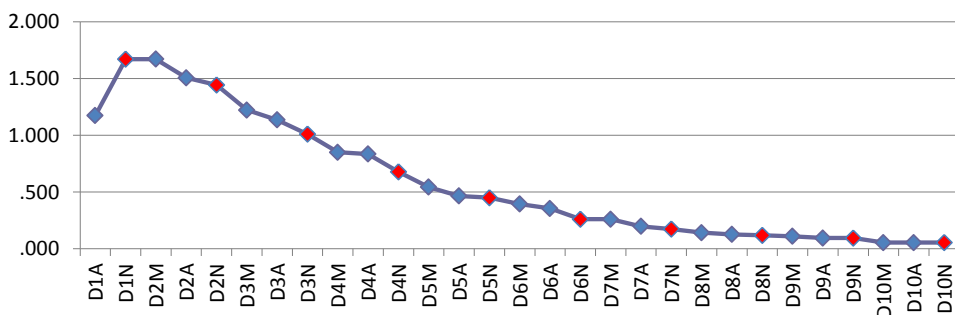
was established that under 0.5 points (approximately 30% of the maximum PL experienced) pain could be considered as slight, between 0.5 and 1.5 as moderate, and above 1.5 (approximately 70% of the maximum PL experienced) as high. These stratification values are shown in figure 7. Using this categorization we can consider that the Ni-Ti archwires cease to hurt from 85h (3.5 days) from beginning of treatment as opposed to 109 hours (4.5 days) for SS archwires.

### 7.1.3. Peak of maximum pain

It is interesting to analyse the moment at which the peak of maximum pain intensity is reached. In our experimental data, the maximum pain is reached in the morning of the day after fitting the archwires, both for Ni-Ti and SS archwires. More specifically, if we observe the fits made (figure 7), maximum pain arrives at between 10 to 12 hours after fitting either of the types of archwire. However, if we consider the peak of the pain when the  $PL \geq 1.5$ , as we commented earlier, we can see that that the peak takes place between 8-36 hours in SS and 8-20 hours in Ni-Ti, being, therefore, longer in patients with SS archwires.

### 7.1.4. Comparison of pain between time periods

Figure 8 represents the average PL for each time period and it can be observed that there are no “pain peaks” associated with night time, although in the first 5 days, during the night time period a slight increase in pain or a lower decrease than that which would correspond to the 8 hours elapsed from the previous afternoon is noted. This has no statistical significance as the increase or decrease in pain is associated more with the number of hours elapsed since fitting the appliance. Indeed, the AVOVA carried out on the PL values for morning, afternoon and night, the results of which can be seen in Table 4, shows that there is no difference between the mean values with  $p > 0.999$ , although a very slightly higher mean value of PL can be appreciated for the night period.



**Figure 8.** Pain level (PL) for each time period, regardless of the archwire used. The points in red correspond to the PL of night time periods.

	Mean PL	SD	IC95%	Minimum	Maximum
Morning	.58	.56	0.15 - 1.01	.06	1.67
Afternoon	.59	.53	0.27 - 0.97	.06	1.51
Night	.60	.59	0.17 - 1.02	.06	1.67

**Table 4.** Mean values and standard deviation (SD) of pain level (PL), confidence interval for the mean of PL at 95% (IC95%) and minimum and maximum values of each time period (morning, afternoon and night), regardless of the type of archwire used.

## 7.2. Subjective study of pain

### 7.2.1. Biochemical mediators analysis

To complete the study of pain, an objective assessment was performed. Two biochemical mediators of pain (Prostaglandin E2 (PGE2) and Substance P (SP)) were determined for each patient during treatment in four time intervals: prior to bracket bonding, 24 hours after bonding, a week and a month after bonding. In all cases, direct measurements were not considered but the proportion to the amount of crevicular fluid collected.

It was analyzed the possible correlations between the subjective pain reported by the patient with the levels of these mediators in the crevicular fluid for all the times studied.

### 7.2.2. Prostaglandin E2 (PGE2).

Correlations have been found between the concentration of PGE2 (PC) in the 4 times in which the determinations of this mediator were taken (PCT1: start, PCT2: 24 hours, PCT3: 7 days and PCT4: 30 days) and the subjective pain level of each individual in each interval analyzed, taking into account the 10 days in which there was existence of subjective pain. The results are shown in Table 5.

		D6N	D7M	D7T	D7N	D8M	D8T
P_Ct1	r-Pearson	0,102	0,274*	,280*	0,288*	,303**	0,140
	Sig. (bilateral)	0,386	0,017	,015	0,012	0,008	0,231
P_Ct2	r-Pearson	-0,032	0,053	,064	0,020	0,029	0,001
	Sig. (bilateral)	0,787	0,650	,583	0,862	0,803	0,993
P_Ct3	r-Pearson	0,010	0,161	,122	0,139	0,273*	0,173
	Sig. (bilateral)	0,931	0,167	,296	0,234	0,018	0,137
P_Ct4	r-Pearson	0,242*	0,326**	0,420**	0,442**	0,252*	0,295*
	Sig. (bilateral)	0,037	0,004	0,000	0,000	0,029	0,010

**Table 5.** Study of the correlation between the level of concentration of PGE2 (PC) in the 4 time times analyzed (PCT1 correspond to the time prior to bracket bonding, PCT2 to 24 hours after, PCT3 a week after and PCT4 a month after) and the level of pain reported by each patient. Values with \* correspond to the times when correlation was found between the two measures of pain.

It can be seen that, in general, individuals with initially high values of PC mediator have a correlation with higher levels of pain one week after the archwire placement. This situation is also shown with PCT3 values and with those of PCT4, with no significant correlation with PCT1.

If the correlation of this mediator is analysed, not with the level of pain but with the total pain experienced by the subjects during the first 10 days, we find that high values of PC are correlated with subjects having more total level of pain throughout the treatment (Table 6). This finding does not happen with this marker in the other periods of time.

		P_Ct1	P_Ct2	P_Ct3	P_Ct4
Total Pain	r- Pearson	0,229*	0,059	0,025	0,166
	Sig. (bilateral)	0,048	0,616	0,831	0,156

**Table 6.** Study of the correlation between the level of PGE2 concentration (PC) and total pain level.

Furthermore, we analyzed whether there was correlation between the values of PGE2 in the four times studied. The results are shown in Table 7 and confirm that initially high values of this mediator are correlated to high values along the entire treatment.

		Total Sum	P_Ct1	P_Ct2	P_Ct3	P_Ct4
P_Ct1	CP	-0,042	1	0,484**	0,352*	0,365*
	SG	0,797		0,002	0,026	0,021
	N	40	40	40	40	40
P_Ct2	CP	-0,037	0,484**	1	0,120	0,297
	SG	0,821	0,002		0,462	0,063
	N	40	40	40	40	40
P_Ct3	CP	-0,268	0,352*	0,120	1	0,318*
	SG	0,094	0,026	0,462		0,046
	N	40	40	40	40	40
P_Ct4	CP	-0,004	0,365*	0,297	0,318*	1
	SG	0,978	0,021	0,063	0,046	
	N		40	40	40	40

**Table 7.** Study of the correlation between the values of prostaglandin PGE2 in the four times tested. CP (Pearson Correlation) and SG (Sig. Bilateral) \*\* significant correlation at the 0.01 level and \* at the 0.05 level (bilateral).

### 7.2.3. Substance P (SP)

In order to analyse this biochemical mediator, 86 cases were taken into account. The mean values of SP in the four times analysed of the study are shown in Table 8 and Figure 9.

Factor 1	Mean	Error	IC 95%	
			Lower limit	Upper limit
SPC1	173,165	21,470	130,469	215,860
SPC2	139,728	20,928	98,111	181,345
SPC3	190,621	26,825	137,278	243,965
SPC4	230,510	45,338	140,351	320,670

Table 8. Mean values of SP for each of the times studied.

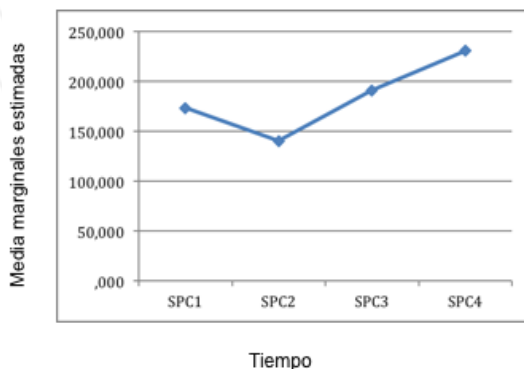


Figure 9. SP concentration at each time for each of the times studied.

According to these data, no statistical difference was found between the objective pain (SP crevicular fluid analysis) with the SS or Ni-Ti archwires, and the total subjective pain experienced according to the questionnaire.

## 8. Further research

In the literature, we have found few studies that compare pain depending on the type of archwire used [5, 32, 35] and none that introduces a mathematical equation for predicting pain level or peak of pain.

### 8.1. Subjective study of pain

#### 8.1.1. Pain level

Our results coincide with those of Lee *et al.* [32] in stating that there is less pain in the group of patients treated with Ni-Ti archwires than in the group treated with SS archwires. However, neither our results nor the previously mentioned ones coincide with those of Jones and Chan



[5], who did not find differences in the pain experienced by patients between Ni-Ti archwires or SS archwires. Moreover, despite being a quite different study, Fernandes et al. [35] also did not find any difference on comparing conventional Ni-Ti archwires with super elastic Ni-Ti archwires.

In our results, pain receded at 4.5 days in cases treated with SS archwires, whereas it receded one day earlier (at 3.5 days) in those cases treated with Ni-Ti archwires. Moreover, the pain level was lower in the group with Ni-Ti archwires than in those with SS archwires.

If we analyse the results of the subjective study of pain recorded by the patients, we observe how 38.2% of patients felt discomfort and 43.1% felt slight pain, whereas only 18.6% experienced intense pain. These results do not coincide with those of Kaneko et al. [36], where only 10.3% felt discomfort and the great majority (72.4%) felt only slight pain. However, their results are in line with ours in stating that 17.2% experienced intense pain.

### *8.1.2. Peak of maximum pain*

If we analyse when the peak of maximum pain takes place, the results of our study show that the peak occurs 15 hours after fitting the archwires into the mouth of the patient, both in the Ni-Ti group and the SS archwire group, results that are similar but not identical to those of other authors [1, 3, 6, 18, 25, 37] who observed a maximum peak of pain 24 hours after fitting the archwire in the patient's mouth.

In contrast, our results and those mentioned above do not coincide with the results of the work of Jones and Chan [5] who found the maximum peak of pain to be on the same morning when fitting the archwire in the mouth, or the results of Jones and Richmond [38] who found the maximum peak of pain in the afternoon or night of the same day that the archwire was fitted.

### *8.1.3. Duration of pain*

On studying how long pain lasts, the results of our study show that pain ceased at 3.5 days in the Ni-Ti group, whereas, in the SS group, it ceased at 4.5 days, the decrease in pain taking place earlier in the Ni-Ti group than in the SS group. These results do not coincide with those of Jones and Richmond [38], Jones and Chan [5], Ngan et al. [4] or Scheurer et al. [6] in whose studies, the pain caused by the fitting of the archwires was observed to last for approximately 5 days.

Our results show how both groups of patients, both the patients fitted with SS archwires and those fitted with Ni-Ti archwires, began to experience pain at 4 hours, results similar to those of Jones and Richmond [38] with the same intensity of pain.

However, SS and Ni-Ti metals do not have the same stress deformation curve and it can be observed that in order to achieve the same deformation, the stress that has to be applied in the case of steel is greater than in the case of Ni-Ti, which would account for the greater initial pain of the patient in cases treated with SS archwires. Nevertheless, as the teeth adapt to the forces applied and, therefore, the stress applied on the archwire material diminishes, these two materials behave differently, SS maintains a residual deformation, whereas Ni-Ti returns to

practically its original dimensions, meaning that its effect on the tooth is more continuous and produces a sensation of pain over a longer time, so evening out the total time of pain duration for both types of archwire.

#### *8.1.4. Comparison of pain between time periods*

In our study, we did not find pain peaks associated with night time, unlike those shown in the results of Kaneko et al. [36] but that the increase or decrease of pain was similar in the morning or afternoon and was only associated with the number of hours following the fitting of the appliance. These results coincide with those of Scheurer et al. [6] who found that pain increased as the day elapsed.

## **8.2. Objective study of pain**

Authors like Erdinic and Dincer [8] or Awawdeh et al. [39] have observed that the perception of pain during orthodontic movement is related to substance P. Substance P is involved in the nervous system signals required to perceive pain [40] also influencing the concentration of other pain mediators associated with dental pain like the metalloproteinase 8 [41], and in the secretion of IL-1 $\beta$  from the monocytes. These three substances (IL-1 $\beta$ , PG-E2 and SP) were independently associated with pain [15-17] and are expressed during initial tooth movement in sufficient amounts to be detected in the crevicular fluid [18].

Then we will discuss some variables affecting the collection of this sample.

#### *8.2.1. Sources of error in the collection of the crevicular fluid*

Before taking the samples, one have to keep in mind that the most frequent sources of contamination of the crevicular fluid samples are blood, saliva and plaque. Respect to the presence of plaque, its presence in the paper strips used for collecting the sample has a considerable effect on the volume of the sample, thus being a source of bias [42-44]. These considerations have been supported also by other authors, who demonstrate that the non-removal of the plaque has a bad effect on the determination of the volume [43,45].

In order to avoid these problems of contamination prior to bracket bonding, scaling was performed to all patients of the study, thus reducing the possibility of bleeding and contamination by plaque.

Also, in our study, in order to reduce saliva contamination, a good insulation protocol was carried out by placing the aspiration system and cotton rolls in each patient. Saliva contamination is a problem when the sample is collected with paper strips, as it can alter the volume of the sample collected [46].

#### *8.2.2. Sample recording time*

The first authors who described the use of Periotron® [43] recommended a sample recording time of five seconds. However, to increase the volume of the sample in order to subsequently analyze it sometimes it is necessary to increase the sample time.

Therefore, in our work, a sample time of 30 seconds was established following the recommendations from other authors [32, 47-50]. For the analysis of the crevicular fluid, the collection should be made in such way that minimum groove environment deterioration occurs in the shortest time possible. By doing so, correct protein concentrations are maintained and also sufficient time is achieved to collect the required sample volume.

### 8.2.3. *Methods of collection of crevicular fluid*

There are many ways to collect the crevicular fluid; gingival washing, micropipettes and paper strips. Gingival washing method is suitable for obtaining cells of the gingival groove. It is a complex method and has limited applications since it can only be used in the maxillary arch due to the complexity of the technique. On the other hand, samples cannot be analyzed later in the laboratory and all the fluid cannot be recovered during aspiration and re-aspiration.

With micropipettes is difficult to collect a necessary amount of fluid in a short period of time unless there is gingival inflammation, resulting in more volume of crevicular fluid. It can cost up to 30 minutes to collect the amount of fluid necessary and this makes this technique traumatic [51].

The crevicular fluid analysis it is a useful and advantageous method, especially for in vivo studies, it is not invasive, and the sample can be split as many times as necessary. This allows the perfect monitor development in a given area for a certain period of time. Therefore and because of the complications mentioned above with the two methods described, in our work we decided to take the sample by this method, using paper strips, like other authors [32, 47-50] because it is a simple, rapid and non-traumatic method and can be applied to isolated areas.

Crevicular fluid samples of each patient who also had filled the subjective pain questionnaire were collected, in order to later compare the subjective data of pain with the objective values obtained from the analysis of the biochemical mediators.

## 8.3. Biochemical mediators analysis

### 8.3.1. *Prostaglandin E2 (PGE2)*

In our study, PGE2 production reached its maximum peak at 24 hours, coinciding with the results found by Bergius et al. [37], Giannopoulou et al. [18] and Grieve et al. [52]. In another study by Lee et al. [32], initial measurements of IL-1  $\beta$  and PGE2 showed little variation, however, once force is applied, the individual variation became large enough to estimate the overall response.

Lee et al. [32] observed an increase in the levels of interleukins in patients during the third week, even without the revive forces, causing an increase in the average concentration but without statistical significance. Iwasaki et al. [30] found that IL-1 $\beta$  levels fluctuate with a period of 28 days when a force is applied continuously.

Interestingly, when a discontinuous force (equivalent to steel arches) is reactivated after a week, a significant increase in the levels of IL-1 $\beta$ , 24 hours after reactivation was found,

compared with the results found 24 hours after the initial activation. This finding implies that timely reactivating discontinuous forces might be more effectively in the regulation of IL-1 $\beta$  than the continuous application of forces. Instead, they did not found any increased after a second reactivation. This could be due to a refractory period or excessive applied pressure. Since excessive pressure causes a large area of hyalinization in the pressure side and a wide acellular area, which produces impairment in cytokine secretion and its spread to the periodontal space. These results suggest that the application of a suitable pressure force intermittent and timely recovery may be effective to promote secretion of IL-1  $\beta$ . However, authors like Yamaguchi et al. [26], Tian et al. [27] or Uematsu et al. [11], argue that the intense and discontinuous forces tend to significantly reduce levels of IL1 $\beta$  suggesting the need for reactivation to maintain sufficient production of IL1 $\beta$ . Such forces not only increase the risk of root resorption but also increase the hyalinization of periodontal tissue [28,29] which also induce very pronounced rise and fall peaks in the levels of cytokines, leading to undesirable tissue level and the need for the reactivation of the forces.

On the contrary, continuous light forces [30-32] tend to maintain high levels of IL1 $\beta$  so the need for reactivation is reduced. These forces maintain, high longer, cytokine levels, which are necessary for continuous periodontal remodeling.

The efficiency on the recovery is shown in a series of experiments in rats done by King et al. [53] where the reactivation during the end of the cycle stimulates bone resorbing osteoclasts and reduce root resorption.

In the study by Lee et al. [32], changes in the levels of PGE2 to mechanical stress and the interactions between the IL-1 $\beta$  and PGE2 in vivo were investigated. The mechanical loads applied to the periodontal ligament cells are known to induce the expression of cyclooxygenase-2 (COX-2), which facilitates the formation PGE2 [54]. In the study of Saito et al. [55], a significant increase in PGE2 was observed when IL-1 $\beta$  was applied to periodontal ligament cells alone or in combination with mechanical stress. The synergistic action of IL-1 $\beta$  and mechanical stress on the production of PGE prove the hypothesis that mechanical stress provides more substrate for cyclooxygenase by activation of phospholipase A2, while IL-1 $\beta$  formation increases cyclooxygenase.

PGE2 levels in this initial study showed significant peaks at 24 hours (T2) of the application of force, compared to the control site. This appears to be a direct effect of mechanical stress. By applying continuous force, high levels of PGE2 was observed only temporarily, compared to the control site, even though the average concentration is maintained at a high level during the experiment. With discontinuous forces, significantly higher levels of PGE2 for 1 week were observed. This regulation is an example of the synergistic effect of the mechanical stresses sustained and secreted IL-1 $\beta$ .

In this study we found that, if the total pain recorded by the subjects is determined and their correlation is sought with the values of this mediator, patients with high values of PC1 have more total level of pain during the treatment. Furthermore, PC3 high values are related with high values one week after the archwire placement. We did not find association between patients who had elevated levels of PC4 with high levels of pain between the 6<sup>th</sup> and 8<sup>th</sup> day.

These results cannot be compared with other authors as there are no studies reported in the literature.

### 8.3.2. Substance P (SP)

The results of the substance P values were inconsistent with what would be expected of a biochemical mediator of pain as, in the initial time of the study, when no level of pain exists (and therefore this substance should have a baseline), we found higher values, both overall levels and concentrations, than in the rest of study for 56 of the 86 patients analyzed.

The apparently anomalous behavior of this marker with a decrease at 24 hours and a later increased tendency to grow up is not statistically significant because, due to the high variability of the data. However, other authors as Giannopoulou et al. [18], found more predictable values in the levels of these biochemical mediators after insertion of ligatures.

The experience of pain has its peak 1 day after starting treatment and is reduced to normal levels at 7 days. The IL-1 $\beta$ , SP, PGE2 mediators are expressed during initial tooth movement. The initial perception of pain (1h) is related to the levels of PGE2, the IL-1 $\beta$  is related to pain at 24 hours and the SP has its peak at 24 hours. This is possible because of the relationship that has with PGE2 as an indicator of periodontal inflammation [56].

On the other hand, Yao et al. [57], did not find relationship between pain intensity and PGE2 at 12, 24 and 72 hours of the beginning of tooth movement. Another authors, though [26], found no relationship between IL-1 $\beta$  and substance P. Indirectly, this finding also has an association between IL-1 $\beta$  and pain.

## 9. Conclusions

This paper has analyzed the pain associated with the placement of the first archwire in the beginning of the orthodontic treatment, analyzing the influence that several variables have (such as the type of wire used) on pain.

A questionnaire for the subjective assessment of pain has been designed, registering the pain indicated by the patient at each time interval (morning, afternoon and evening), from the time of placing the archwire for the first time until it disappears. Furthermore, the objective assessment of pain has been determined in the crevicular fluid through the different biochemical mediators of pain, such as prostaglandin 2 (PGE2) and substance P (SP).

- Total pain (TP) and the maximum level of pain (PL) are lower in the group of patients fitted with Ni-Ti archwires than in the group fitted with SS archwires, although pain for both groups recedes at the same time.
- Pain level (PL) is determined by the mathematical equation:

$PL = a * T^{-3+c} * \exp(-b * T)$  where parameters a, b and c represent: "a" coefficient represents the higher or lower PL corresponding to the "peak". The "b" parameter indicates the greater or lesser speed in the reduction of pain and value "c" represents the higher or lower PL throughout the treatment.

- Both the patients with SS archwires and those with Ni-Ti archwires began to feel pain after 4 hours of the placement of the archwires. The maximum peak of pain is established generally within the first hours of the placement of the archwire (10 hours) and lasts for about 20 hours. The pain becomes moderate and mild, respectively, about one and a half (30 hours) and 4 days (100 hours) after the beginning of the treatment. Pain disappears generally before 10 days after the placement of the archwire. There is no difference in the time when pain is greatest for both types of archwire, being situated at between 15 and 20 hours following the fitting of the archwire in the mouth.
- Biochemical mediators (prostaglandin E2 (PGE2) and substance P (SP)) have not reported generally good correlation with the subjective pain reported by the patient. Only it should be noted that high values of PGE2 before treatment (PCT1), correlate with higher levels of subjective pain a week after the archwire is placed. This situation is also shown in the values determined after one week (PCT3) and after a month (PCT4).

## Author details

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