

Original Article

Journal of Medicinal and Chemical Sciences

Journal homepage: <u>http://www.jmchemsci.com/</u>



Evaluation of Pain between Double Slot Orthodontic Brackets Versus Single Slot by Visual Analogue Scale and Saliva Biomarkers

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ARTICLE INFO

Article history

Receive: 2023-01-08 Received in revised: 2023-03-30 Accepted: 2023-04-06 Manuscript ID: JMCS-2303-1978 Checked for Plagiarism: **Yes** Language Editor: Dr. Fatima Ramezani Editor who approved publication: Dr. Nenad Ignjatovic

DOI:10.26655/JMCHEMSCI.2023.10.3

K E Y W O R D S Brackets Double slot Orthodontic Pain Swelling

Visual analogue scale

ABSTRACT

Brackets and various arch-wires are used in fixed orthodontic appliances to apply forces to the teeth. The amino-acid glutamate is one neurotransmitter that has been discussed in the context of pain processing and has been linked to a variety of pain syndromes. This study aims to evaluate pain between Double slot orthodontic brackets versus single slot with a visual scale and a saliva biomarker (glutamate testing) and CRP. This randomized clinical trial (double-blind) was conducted in Dentistry/Howler Medical University. 41 patients (26 females and 16 males) aged of 18-23 years old with class II malocclusion were selected from patients seeking orthodontic treatment after proper diagnosing using digital cephalometry, OPG. The patients who were chosen randomly received either a single arch-wire (0.014 NiTi) or a double arch-wire (one 0,014 NiTi and the second 0.012 NiTi). Two saliva samples were prepared and analysed to measure glutamate and CRP and evaluate pain using the visual analogue scale. The mean visual scale was 4.38 in the twowire group compared to the single-wire cases, which was 2.80 showing a significant difference ($P \le 0.001$). There was a significant statistical difference between glutamate after and before treatment for single and double-slot bracket cases. There was a significant difference in mean pain scores between two group with less pain in single wire with a Visual analogue scale, but there is an insignificant difference with saliva glutamate measurement. However, the levels of glutamate before and after applying force were significant in both groups.



GRAPHICALABSTRACT



and CRP

ariables	Mean	Ν	Std. Deviation	p-value	t-test	
Glutamate before	3.2195	20	0.475	0.026	Significant	
Glutamate after	3.5025	20	0.598	01020		
CRP before	0.0065	20	0.009	0.356	Non-significant	
CRP after	0.0035	20	0.008			
Variables			a or acaute stor		and and a	
Variables						
C1 / / 1 C	Mean	Ν	Std. Deviation	p-value	t-test	
Glutamate before	Mean 3.166	N 21	Std. Deviation 0.559	p-value <0.001	t-test Highly significa	
Glutamate before Glutamate after	Mean 3.166 3.703	N 21 21	Std. Deviation 0.559 0.535	p-value <0.001	t-test Highly significa	
Glutamate before Glutamate after CRP before	Mean 3.166 3.703 0.008	N 21 21 21 21	Std. Deviation 0.559 0.535 0.012	p-value <0.001 0.188	t-test Highly significa Non-significa	

Glutamate and CRP of single slot bracket cases alone

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Introduction

Orthodontics is a dentistry specialty that addresses the diagnosis, prevention, management, and correction of mal-positioned teeth and jaws, and misaligned bite patterns [1]. In permanent orthodontic appliances, pressures are applied to the teeth using brackets and different types of arch-wires. To move teeth with minimal patient discomfort and pathological effects on the teeth and their supporting structures, light and continuous forces are needed [2]. Biologically adequate forces enable tooth stimulation to happen with few negative effects. Nevertheless, inappropriate pressures may result in increased periodontal ligament (PL) stress, which can lead to hyalinized areas, delayed tooth movement, pain discomfort, tooth mobility, bone loss, and root resorption [3].

Pain is a significant physiological and emotional experience, and the degree to which it manifests itself depends on some factors, including age, sex, emotional state, cultural context, and pain experience. Almost 95% of orthodontic patients have complained of different levels of discomfort [4]. The study by Diddige et al. (2020) has shown that pain intensity increases between four and twenty-four hours following the start of orthodontic treatment, stay high for two to three days, and then progressively returns to baseline by the seventh day [5]. It is challenging to objectively express a subjective sensation like pain. Typically, tools such as the visual analogue scale (VAS), numerical rating scale (NRS), or verbal rating scale are used to measure the intensity of the pain. These tests are only partially useful because patients' responses are impacted by their surroundings, how they perceive pain, and how long they expect the pain to last [6]. Researchers' efforts to discover a suitable platform for the objective evaluation of biomarkers linked to pain, discomfort, or stress throughout time have increased interest in salivary diagnostics [7].

Recently, many neuropeptides from the whole and glandular saliva of healthy people were successfully identified and analysed [7]. Neurons frequently communicate with one another through neuropeptides, which are tiny chemicals secreted from brain cells. Co-release of some occurs with other neuropeptides neurotransmitters. Neuropeptides serve а number of important roles besides their primary role as neurotransmitters [8]. The amino-acid glutamate is one neurotransmitter that has been discussed in the context of pain processing. Its increased level by painful stimuli and found in both central and peripheral nerve terminals. As a result, it has been linked to various pain syndromes [9, 10]. CRP has been widely used as a robust inflammatory biomarker for many health conditions in both clinical and research settings, and several studies have shown a positive correlation between plasma CRP levels and pain intensity [11].

Banerjee et al. (2018), in their study t found that pain is one of the important consequences of orthodontic treatment and has a significant impact on life quality of orthodontic patients, especially in the early stages of treatment [12]. So, to minimize these negative consequences, it is necessary to pay special attention to the pain caused by orthodontic treatment. Therefore, this randomized clinical trial's objective was to compare the levels of pain and discomfort caused by fixed appliances with new bracket design (double slot) with two initial arch-wires for levelling, alignment, and single slot brackets (conventional) with single initial arch-wire by measuring with a visual scale and a saliva biomarker (glutamate testing) and CRP (Creactive protein) both before starting the fixed appliance and 48 hours after it had been applied.

Martials and Methods

This randomized clinical trial (observational study) was conducted in the Departments of Pedodontics, Orthodontics, and Preventive Dentistry/College of Dentistry/Hawler Medical University. The clinical trial was registered by SLCTR/2022/015.

The study aimed to investigate the levels of discomfort or pain with the initial arch-wire for leveling and alignment during fixed orthodontic treatment. In this regard, from single-slot open horizontal orthodontic brackets (with a 0.014" NiTi arch-wire in the initial stage for alignment)

(Figure 1), and from two-slot orthodontic brackets (a new design with 0.014" Niti in the occlusal slot and 0.012" NiTi on the gingival slot) (Figure 2) were used. For standardization, the same orthodontic Brackets were used for all patients (Sortech Company).

In the current research, 41 patients (26 females and 16 males) between the ages of 18 and 23 years old with class II malocclusion (overjet less than 6 mm and over bite between 4 and 0 mm) were selected from patients seeking orthodontic treatment after proper diagnosing using digital cephalometry, OPG, intraoral and extraoral photo, an intraoral scan. All participants gave their consent in writing after being fully informed. 42 coded packets (22 DW=double wire and 22 SW=single wire) were placed in a box. After mixing, the patients randomly and with closed selection were placed in two groups.

Patients did not know which group they belonged to. The treating orthodontist was also partly blinded between groups because he did not know which envelopes contained double wires and which contained single wires. However, because of the therapy nature, the treating orthodontist was aware of whether the patient was allocated to DW or SW. Furthermore, the laboratories contained no details about the study groups.



Figure 1: Using a single slot with one initial arch-wire (0.014 NiTi)



Figure 2: Double slot brackets with double wire 0.014 NiTi on the occlusal slot and 0.012 on the gingival slot.

Class II malocclusion, overjet less than 6 mm, crowding index of mild to moderate disorganization, absence of systematic diseases, and adequate dental hygiene were the inclusion criteria. Likewise, the criterion for exclusions were individuals who missing teeth and have impacted teeth (except the 3rd molar) patients with significant oral hygiene issues, those who reported using drugs throughout the research, and those who have had orthodontic treatment in the past.

The patients who were chosen randomly to receive either a single arch-wire (0.014 NiTi) or a double arch-wire (first 0,014 NiTi and second 0.012 NiTi). After the arch-wire was put in place, they were all tied to the teeth with the same elastic ligature (3M Company) and instruction gave to all patients not drink too hot and too cold drinks until return to the clinic after 48 hours.

They were treated with the same standard procedures, by the same clinician, and at the same clinic. The treated malocclusions had similar characteristics: slight or moderate overcrowding treated without dental extractions.

Saliva sampling and analysis

Volunteers were asked to refrain from eating and drinking, at least one hour before saliva collection. All saliva samples were collected in the same order, in the same clinical room, and at the same time, between 9 and 11 a.m. The saliva of each patient was collected before bonding brackets and 48 hours after applying orthodontic force by initial NiTi arch-wires. This was collected using a disposable pipette and stored in sterile microcentrifuge tubes for subsequent analysis participants were required to spit The samples were transported to the laboratory in a Styrofoam container with ice for glutamate measurement and C-Reactive Protein (CRP). After 48 hours, each patient returned to the clinic for the 2nd sample of saliva collection and assessment of pain using the Visual Analogue Scale (VAS).

Salivary Glutamate with CRP in each sample was measured using Human Glutamate Elisa Kit (SunLong Biotech Co. LTD).

Results and Discussion

We enrolled 41 patients in the current study, divided into two groups. Twenty patients have been randomly chosen and treated with single slot bracket wire (single wire cases) and for the rest 21 patients, the double slot bracket wires were applied (double wire cases). Generally, most (63.4%) of cases were female and 36.6% were male, as presented in Figure 3.

Table 1 indicates that the mean visual scale for pain ± (SD) of participants was $3.61 \pm (1.09)$, the mean glutamate before the procedure of applying the wires ± (SD) of patients was $3.19 \pm (0.51)$ µmol/L while the mean ± (S.D) glutamate after the procedure increased to $3.60 \pm (0.569)$ µmol/L. The mean CRP before treatment or procedure ± (S.D) of the whole sample size was $0.007 \pm (0.011)$ mg/dL and finally, the mean CRP after treatment ± (S.D) of samples sizes was $0.003 \pm (0.008)$ mg/dL.



Figure 3: Participants sex.

Variables	Ν	Range	Minimum	Maximum	Mean	SD
Visual scale	41	5	1	6	3.61	1.09
Glutamate before (µmol/L)	41	1.91	2.42	4.33	3.19	0.514
Glutamate after (µmol/L)	41	2.28	2.64	4.92	3.60	0.569
CRP before (mg/dl)	41	0.04	0	0.04	0.007	0.011
CRP after (mg/dl)	41	0.04	0	0.04	0.003	0.008

Table 1: Mean, standard deviation, range, the minimum and the maximum of numerical variables for the whole

Table 2: Correlation between glutamate after procedure and visual scale

		Visual scale	Glutamate after
	Pearson Correlation	1	0.416**
Visual scale	Sig. (2-tailed)		0.007
	Ν	41	41

**Correlation is significant at the 0.01 level (2-tailed).



Figure 4: Correlation between glutamate after procedure and visual scale

According to Table 2 and Figure 4, there was a positive moderate correlation between the visual scale for pain and glutamate level, i.e. with an increase in glutamate concentration in blood, the visual scale would moderately increase. The correlation was statistically significant (P \leq 0.007).

As indicated in Table 3, there was a nonsignificant difference between study groups and glutamate before the procedure ($P \le 0.745$). The cases treated with single wire had a mean glutamate of 3.219 μ mol/L. Similarly the mean glutamate of double wire cases was 3.166 μ mol/L.

There was a non-significant difference between study groups and glutamate even after the procedure (P \leq 0.263). Double wire cases had mean glutamate of 3.703 µmol/L, in the same way, the single wire cases had the mean glutamate of 3.502 µmol/L.

There was a significant difference between study groups and the visual scale (P < 0.001); Double-

wire patients defined the situation as an unpleasant and more painful procedure (the mean the visual scale of 4.38) in comparison to single wire cases who recorded only 2.80 scores as the mean visual scale (Table 3).

Single slot bracket cases alone

The findings of Table 4 reveal that there was a significant difference between glutamate after

and before treatment for single-slot bracket cases (P \leq 0.026). The mean glutamate before the procedure was 3.5025 µmol/L while the mean glutamate of the same cases had increased to 3.2195 µmol/L after applying the single wire slot bracket was significant. In contrast, there was a non-significant difference (P \leq 0.356) between CRP before (mean 0.0065 mg/dl) and after treatment (mean 0.0035 mg/dl) (Table 4).

Table 3: Difference in visual scale	and glutamate for 1	measuring pain betwee	en single and dou	ble wire groups
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Variables	Study groups	Ν	Mean	Std.	P-value	T-test
Clutamata hafara	single wire	20	3.219	0.475	0.745	Non-significant
Giutalliate Delore	double wire	21	3.166	0.559	0.745	
Chatana ta aftan	single wire	20	3.502	0.598	0.263	Non-significant
Giulamale allei	double wire	21	3.703	0.535		
Visual scalo	single wire	20	2.80	0.696	< 0.001	Highly significant
VISUAI SCALE	double wire	21	4.38	0.805		

Table 4: Glutamate and CRP of single slot bracket cases alone

Variables	Mean	N	Std.	P-value	T-test			
Glutamate before	3.2195	20	0.475	0.026	Significant			
Glutamate after	3.5025	20	0.598		Significant			
CRP before	0.0065	20	0.009	0.356	Non significant			
CRP after	0.0035	20	0.008	0.330	Non-Significant			

Outcomes of Table 5 indicate that there was a significant difference between glutamate after and before treatment ($P \le 0.001$). The mean glutamate before treatment was 3.166 µmol/L while the mean glutamate of the same cases had augmented to reach a mean of 3.703 µmol/L after applying the double wire slot bracket, t-test was done. In contrast, there was a non-significant statistical difference ($P \le 0.001$) between CRP before (mean 0.008 mg/dl) and after treatment (mean 0.003 mg/dl) and p-value was 0.188 (Table 5).

Discussion

The most prevalent and troublesome side effect of orthodontic therapy is pain and discomfort. During fixed orthodontic therapy, 87% to 95% of patient report discomfort, particularly during the first 24 hours [12, 13]. Furthermore, 39%-49% of patients report discomfort at every stage of therapy or after the appliance is removed. As a result, pain is a significant barrier to orthodontic treatment, а factor that lowers patient compliance during treatment, and a reason why patients stop treatment or skip meetings. Orthodontic therapy is painful for 90% of patients and 30% may discontinue treatment early due to discomfort [12]. This observational study analysed perceived pain after the initial placement of two different pre-adjusted fixed appliance systems. The pain was measured using a VAS, which is one of the most commonly used tools in the measurement of perceived discomfort during orthodontic treatment. This system is readily understood by most patients and is reliable [14]. For more confirmation also pain was compared by saliva biomarker with glutamate measurement before and after applying the force of fixed appliance since glutamate gamma-aminobutyric acid is one of the (pain-producing substance) algogenic like substances p it is released during orthodontic tooth movement [15]. Canton- Habas et al. (2021), mentioned the concentrations of pain biomarkers in saliva, would be an objective tool of enormous utility to confirm the possible diagnosis of pain in this population [16].

The results of examining the sex variable showed that most participants are female. This result was

not consistent with the study of Chouinard *et al.* [17], because the ratio of men and women was the same, but the study by Zheng *et al.* [18], was consistent with our study.

Variables	Mean	Ν	Std.	P-value	T-test
Glutamate before	3.166	21	0.559	<0.001	Highly significant
Glutamate after	3.703	21	0.535	<0.001	
CRP before	0.008	21	0.012	0.188	Non significant
CRP after	0.003	21	0.009	0.100	Non-Significant

Table 5: Glutamate and CRP of double slot bracket cases alone

Orthodontic tooth movement is known to trigger inflammatory responses in the oral pulp and periodontium, which will trigger the release of different biochemical messengers that cause discomfort [19]. According to the research, substance P, histamine, enkephalin, dopamine, serotonin, glycine, glutamate gammaaminobutyric acid, PGEs, leukotrienes, and cytokines are among the substances released and presented during orthodontic pain. Orthodontic pain is further believed to be caused by changes in blood flow brought on by the tools [20, 21]. Glutamate is considered to be the main excitatory molecule in the brain, and it is used as the primary neurotransmitter by approximately 60% of the brain synapses. Glutamate receptor action is required for rapid synaptic transmission, synaptic plasticity, learning, memory, muscle coordination, and pain transmission. Therefore, with the increase in glutamate level, the amount of perceived pain will further increase [22, 23]. The results of the comparison variable between pain visual scale and glutamate level in our study showed that there is a statistically significant relationship between these two variables. Because with the increase of glutamate level, the vision scale also increases which confirms these findings. In general, the amount of measured glutamate in both groups before and after applying orthodontic force was very significant. It is an excellent biomarker for assessing pain and utility similar to VAS, and it is readily obtained from saliva without any intervention.

The main factors associated with the discomfort experienced by orthodontic patients are the type of appliance, amount of force applied in the early stages of treatment, previous experiences with pain, and emotional, cognitive, and environmental aspects such as culture, sex, and age [24]. It is a common belief that there is a direct relationship between the amount of force applied to a tooth and the amount of pain a patient would perceive. Though some studies have shown a positive correlation between both variables [25, 26].

The result of the present study showed a statistically significant difference in pain score with a visual scale between the two appliance types with double arch-wire causing more discomfort than single wire and this is agree with Luppanapornlarp *et al.* [26], and Singh *et al.* [25]. But when we compare both appliances for pain perception with glutamate testing statistically non-significant. However, patients were able to endure twin slot brackets with double wire, and this pain can be managed with analgesics.

For double slot bracket with the double wire, we set up force nearly to double for levelling and alignment of teeth when we compare with the conventional bracket with single wire at the same sizes, but the force does not add to double this may be due to we not add force at the same point of force application with double slot brackets. Therefore, in physics called a parallel coplanar force system consists of two or more forces whose lines of action are parallel, this is commonly the situation when simple beams are analysed under gravity loads [27]. We have two points of application of force with two different centres of resistance vertically. Hence, the force added by the tow wire is not like force added by increasing the wire size that's why the pain level was not like directly increasing the force by increasing the size of wires as we can add more force with lesser pain level.

C-Reactive Protein (CRP), is one of the indicators that have a high potential to be tested in saliva rather than plasma to identify and track numerous various systemic and oral diseases. Since CRP is typically immediately transmitted from blood to saliva and has demonstrated a strong correlation with plasma levels in numerous studies, measuring salivary CRP levels rather than plasma levels is a novel and practical approach [28, 29]. Several medical situations, including systemic inflammation, infection, myocardial ischemia, autoimmune diseases, dental inflammation, periodontitis, and sepsis, resulting in a marked rise in this protein in plasma and saliva [28]. The results of examining the C-reactive protein variable in saliva samples showed that CRP has nothing to do with the pain level of. However, there was no statistically significant difference between CRP before (mean 0.008 mg/dl) and after applying orthodontic force (mean 0.003 mg/dL) (p-value=0.188). This was in agreement with the study of Stiimer and Raum *et al.* [30].

Conclusion

The results of the present study showed that there was a significant difference in mean pain scores between the two groups with less pain in single wire with Visual analogue scale, but there is an insignificant difference with saliva glutamate measurement. However, the glutamate levels before and after applying force was significant in both groups. Saliva C-Reactive protein not changed before and after applying force (non-significant).

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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HOW TO CITE THIS ARTICLE

Muhammed Hussein Saleh, Bayan Abdulla Hasan. Evaluation of Pain between Double Slot Orthodontic Brackets versus Single Slot by Visual Analogue Scale and Saliva Biomarkers. *J. Med. Chem. Sci.*, 2023, 6(10) 2282-2291 DOI: <u>http://dx.doi.org/10.26655/IMCHEMSCI.2023.10.3</u> URL: <u>http://www.jmchemsci.com/article 170759.html</u>