Effect of Low Level Laser Therapy on Pain Reduction After Midpalatal Expansion in Rats

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INTRODUCTION

Pain is inevitable during orthodontic treatment. Orthodontists have always tried to minimize pain and discomfort. Due to fear of pain after placement of appliances, many patients may prefer other therapeutic approaches or even reject treatment [1-6]. Previous studies indicate that 90-95% of orthodontic patients report pain during treatment period [2,7]. Orthodontic pain is due to forces applied on the teeth, which cause edema in periodontal tissues, resulting in release of inflammatory mediators [1,8].
Orthodontists use a variety of methods to alleviate orthodontic pain, including cognitive behavioral therapy (CBT) [2], use of non-steroidal anti-inflammatory drugs (NSAIDs) [1-3,7,9], anesthetic gels [1,2], chewing gums and wafer bytes [1,2], electrical nerve stimulation and vibratory stimulation [1]. All the afore-mentioned methods have merits and demerits. Although NSAIDs reduce orthodontic pain, they have side effects such as allergy, peptic ulcer, congestive heart problems and most importantly, they may negatively affect the process of tooth movement by inhibition of bone resorption. These side effects question the use of these drugs [1,2]. Low level laser therapy has been used in dentistry since 1970 and there is evidence of its pain controlling potential and anti-inflammatory properties due to its regenerative effect on neurons [1-3,6]. It is defined as laser treatment with low output power causing insignificant thermal changes and biomodulatory effects [1,2,4,5]. It has been demonstrated that LLLT can postpone the onset and reduce the severity and duration of pain; also it has very few side effects [1]. However, a number of reports have shown that laser therapy has no effect on the onset or severity of pain compared to the placebo group [3]. In order to standardize pain measurement, many studies have used visual analog scale (VAS), which is a psychometric response scale. When responding to a VAS, respondents specify their level of pain by indicating a position along a continuous line between two endpoints, which are no pain as number 0 and worst possible pain as number 10. However, studies on animal models for evaluation of pain and tooth movement cannot use VAS and thus, pain measurement is done through observing animal behavior [4,10,11]. The purpose of this study was to evaluate the efficacy of LLLT for pain reduction after midpalatal expansion in rats by monitoring the body weight of animals during the treatment period.

MATERIALS AND METHODS
In this animal study, 60 six-week old Sprague rats weighing 180± 10g (Razi Vaccine and Serum Research Institute, Tehran, Iran) were used. To standardize the conditions, they were kept in Razi Institute for one week and then moved to the animal house of the Veterinary Faculty of Tehran University (survey site) under similar conditions in terms of water and food supply at 23°C [12]. Rats were monitored after their transfer and were randomly divided into three groups of two experimental (n=24) and one control (n=12) group as follows:

A: Control group: Without expansion or laser therapy
B: Experimental groups:
(a) Expansion, without laser therapy for 14 days
(b) Expansion, with laser therapy for 14 days

Orthodontic intervention:
All rats except for the control group were deeply sedated by intramuscular injection of 10% animal ketamine and 2% xylazine in order to place orthodontic appliances for maxillary expansion. The protocol by Saito and Shimizu [13] was slightly modified and followed for opening the midpalatal suture. This method involved using a 1.5 mm thick stainless steel ring (made of 0.5 mm thick orthodontic wire by an orthodontic technician)(Fig. 1). An initial space, smaller than ring diameter, was created with an osteotome between the incisors. Then the ring was pushed with a slight pressure into the created space for expansion. A hole was created distal to both central incisors along the gingival papillae, in order to keep the ring tight in place, using a straight handpiece with a maximum speed of 35,000 rpm and a ½ round bur. After ring placement, it was fixed using a brass wire with a round cross-section and a diameter of 0.5 mm through the created holes. The aim of this operation was to achieve 1.5mm expansion of midpalatal suture, equal to the
size of the ring used in between the anterior teeth.
At the end, the fixing wire was bent so that it did not irritate the soft palatal tissue of rats.
According to Saito and Shimizu [13] describing the appliance placement in between incisors of rats, initial holes were created in proximal surfaces of incisors and then a 1.5 mm appliance was placed between the teeth (Fig. 2). Pilot assessments revealed that this process can highly make the teeth susceptible to fracture, and this was due to the location of holes adjacent to gingival papilla.
Hence, a slight change was made to make the process safer.
Primarily, the required space was provided by putting the appliance in between the incisors; afterwards, two holes along the long axis of the appliances were created on either side of the incisors. These changes minimized the risk of tooth fracture.

**Laser therapy:**
In this study, Photon-Lase III (DMC, Sao Paulo, Brazil) was used (Fig. 3). The rats received Gallium-Aluminum-Arsenide (GaAlAs) diode laser irradiation with 810 nm wavelength and output power of 100 mW and energy density of 4 J/cm², considering the safety regulations, on days 0, 2, 4, 6, 8, 10, 12 and 14 on four points, with the 0.6 mm optical fiber in contact with the mucosa. Irradiation was performed under general anesthesia. Irradiated points were: A buccal point 0.5 mm apical to the bone crest and three points in the palatal at 1, 2 and 3 mm apical to the bone crest. The non-lased group was generally anesthetized in the same periods of time as the laser treated group in order to have similar anesthetic intervention in both groups. Statistical analysis was performed using one-way repeated measures ANOVA. The results were obtained by weighing the rats daily.
RESULTS
The mean (± standard deviation) weight in all groups before and after treatment is summarized in Tables 1 and 2 and Fig. 4.

The groups had significant differences in terms of weight (P=0.008). The difference in weight of the control and intervention groups was significant (P≤0.05). But there was no difference in weight change between the two intervention groups (P=0.461). All experimental groups wearing the appliance exhibited a weight loss within the first two days. The weight loss in the laser-treated group was significantly less than the value in the non-lased group.

Table 1. The mean weight (gr) of groups during the weighing sessions

| Mean weight (gr) | Day 0   | Day 2   | Day 4   | Day 6   | Day 8   | Day 10  | Day 12  | Day 14  |
|------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Control          | 170.2±32.5 | 174.2±33.1 | 177±35.7 | 178.8±33.5 | 181.2±31.6 | 183.2±20.1 | 187.6±28.7 | 191±28.4 |
| Experimental non-lased | 203.3±28.5 | 192.7±28.3 | 191.4±28.0 | 194.2±27.7 | 200.4±28.3 | 207.8±28 | 210.7±27.5 | 213.3±27.2 |
| Experimental lased  | 180.5±32.2 | 172.2±31.8 | 173.6±31.5 | 176±31 | 180.0±30.8 | 182.0±31.2 | 184.6±30.9 | 185.5±30.5 |

Table 2. The mean weight changes (gr) among the groups during the weighing sessions

| Change in weight (gr) | Days 0-2 | Days 2-4 | Days 4-6 | Days 6-8 | Days 8-10 | Days 10-12 | Days 12-14 |
|-----------------------|----------|----------|----------|----------|-----------|------------|------------|
| Control               | +4±0.8   | +2.8±0.8 | +1.8±0.5 | +2.3±0.6 | +2±0.6    | +4.4±0.6   | +3.4±0.6   |
| Experimental non-lased| -10.5±1.5 | -1.3±1   | +2.8±0.8 | +6.2±0.8 | +7.3±0.8  | +2.9±0.4   | +2.5±0.4   |
| Experimental lased    | -8.2±1.3 | +1.4±0.9 | +2.3±0.8 | +4.0±0.8 | +2±0.7    | +2.5±0.7   | +0.9±0.2   |
Weight loss in laser-treated group turned into weight gain after the second day. However, in the non-lased experimental group weight loss continued until day four. Despite the fact that the non-lased experimental group started to compensate the weight loss after day four, the laser-treated group still showed a significant difference in weight gain in the same period of time, compared to the non-lased experimental group. At the end of day eight, all the rats in laser treated group fully compensated their weight loss. However, this process took 10 days for the non-lased experimental group. Weight change after day eight in the laser treated group was greater than that in the non-lased experimental group, but not significantly (Table 1).

**DISCUSSION**

Pain is one of the most important reasons, which discourages patients from seeking orthodontic treatment. It is believed that pain caused by appliance placement in orthodontic treatment cannot be accurately and reliably measured. In 1962, Burstone [14] introduced a scale for measuring pain; however due to physical and mental differences among people and their impact on pain processing and perception, these scales are not much reliable [15].

However, the effect of pain on life habits such as chewing and eating food is obvious and measurable. One method for measuring the quantity and quality of eating is regular weight measurement, which was used in the current study. Sprague rats were used in the current study, which are a common breed similar to Wistar in animal studies. Only male rats were chosen in order to eliminate the possible confounding effect of hormonal changes on the results. In our study, weight loss was seen during the first two days in both intervention groups. This weight loss, attributed to postoperative pain, could also be due to difficulty eating dry food while wearing the appliance.

However, continuous weight loss in the non-lased experimental group indicates presence of a factor not seen in the laser-treated group. Considering the effects of LLLT on pain reduction through various means, pain is thought to be the distinct factor preventing the rats in the non-lased experimental group from eating properly. Although the reason for pain encountered during rapid palatal expansion is not fully understood, various concepts have been discussed. In this study a special method for rapid midpalatal expansion was used which includes involvement of periodontal ligament (PDL) of both maxillary incisors and the midpalatal suture. Thus, the pain caused by the appliance insertion has both periodontal and osseous origins. Furstman and Bernick [16] in 1972 suggested that periodontal pain is caused by pressure ischemia, inflammation and edema. Burstone [14] identified immediate and delayed pain response, the former being related to the initial compression of the periodontal ligament immediately after placement of the appliance and the latter, which started a few hours later, was termed hyperalgesia of the PDL. Prostaglandins have been shown to cause hyperalgesia, which is increased sensitivity to noxious agents such as histamine, bradykinin, serotonin and substance P. Perception of pain is due to changes in blood flow of the PDL and is correlated with the presence of substances such as prostaglandins and substance P. Factors involved in pain development in PDL can be somehow attributed to bone pain and include factors such as protons, kinins, histamine and serotonin [17]. However, LLLT can affect cell membrane permeability to calcium, sodium and potassium ions leading to events that ultimately result in pain control. These events include degradation of bradykinin [18], increased activity of cellular receptors responsible for inducing production of endorphins [19], decreased activity of C fibers and increased action potential of neurons [18]. This is why various studies have reported LLLT to have...
pain relieving and anti-inflammatory properties [18, 20, 21].

Weight loss of rats in the initial days after placing the appliance is probably due to pain from their rapid maxillary expansion and as mentioned earlier, laser irradiation decreases inflammation, edema and pain. Lasers cause such effects through several mechanisms such as increasing the levels of certain prostaglandins such as PGI2, which have anti-inflammatory effects, increasing the levels of immunoglobulins and lymphokines, which play a role in the immune system and increasing beta-endorphins that are involved in analgesia, inhibition of bradykinin synthesis, doubling the lymphatic drainage and balancing osmotic and oncotic pressure [22]. Significant difference in weight changes within the first few days after insertion of appliance among the groups confirms it. However, as time passed and the critical phase of inflammation caused by the appliance subsided, the level of inflammatory cytokines decreased. Hence, within the second week after orthodontic treatment we noticed regain of weight in the non-lased experimental group rats.

Analgesic effects of lasers have been confirmed in several clinical studies. Lim et al. [23] in 1995, Trotamano et al. [5] in 2009 and Artes-Ribas et al. [9] in 2013 have shown that irradiation of low level laser can reduce pain in patients receiving orthodontic treatment. In the current study, early relief of pain and lower pain peak in days one and two were noted in the laser-treated group. It is worth bearing in mind that in all the afore-mentioned studies, lasers with 830 nm wavelength were used; but in our study, 810 nm wavelength was utilized. It must be underlined that Esper et al. [7] in a study on the effect of LLLT and LED irradiation on pain following orthodontic treatment found that LED was the best choice for this purpose (superior to LLLT). They used 660nm InGaAIP laser for LLLT and 640nm LED. The results can be related to the wavelength used in their study, which affects the superficial parts of tissues.

CONCLUSION

These results show that irradiated group continued to gain weight easier than the control group. This result can be due to more efficient pain control after midpalatal expansion in the irradiated group. After day 14, the conditions of both groups were similar.

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