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ORIGINAL ARTICLE

# Effects of diode laser (980 nm) on orthodontic tooth movement and interleukin 6 levels in gingival crevicular fluid in female subjects

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**Abstract** The aim of this study was to evaluate the effects of low-level laser irradiation on the rate of orthodontic tooth movement (OTM) and the interleukin-6 (IL-6) concentration in gingival crevicular fluid (GCF) during orthodontic treatment. In this randomized split-mouth double blind clinical trial, 11 female patients aged 14 to 25 years (mean 19 ±4.21 year), who required canine retraction following four first premolars extraction, were selected. The GaAlAs laser diode laser (A.R.C. Laser GmbH, Nürnberg, Germany) (980 nm, 100 mW, 5.6 J/cm$^2$, three points from the buccal side and three from lingual side of the tooth, 56 s, running in continuous mode) was used for canine retraction in only one maxillary quadrant (LG). The irradiation time for each cervical and middle third of the tooth was 10 s, and 8 s for the apical third of the tooth. The other maxillary quadrant served as the control group (CG) using the laser pseudo-application in this side. The laser irradiation was applied on days 0, 7, 14, 21, and 28 of each month during the canine retraction phase. Canine retraction was done using closed coil spring with 150 g force on rectangular wires after the alignment and leveling. This study was done in 11 months. Dental casts were made at different time points during the treatment, and the amount of tooth movement was measured. To evaluate the levels of IL-6, GCF samples were collected from the distal side of the maxillary canine teeth on both quadrants at the beginning of the trial, the end of aligning phase, and on day 21 of each month during canine retraction. Although the mean rate of canine retraction was higher in the LG (0.013) than the CG (0.012) and there was definitely a tendency for more canine retraction in the LLLI, but the results failed to show any significant difference between the mean rate of canine retraction of both groups ($P = 0.068$). A paired $t$ test showed that there was no significant difference in the mean concentration of IL-6 at various stages of the treatment between the groups during canine distalization ($P > 0.05$). Therefore, conclusive evidence could not be provided to support the efficacy of the diode laser (980 nm) in accelerating OTM in female subject.

**Keywords** Orthodontic tooth movement · Low-level laser irradiation · Canine retraction · Interleukin-6 · Gingival crevicular fluid

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## Introduction

The time needed for a fixed orthodontic treatment to accomplish is approximately 2–3 years [1]. Prolonged orthodontic treatment can impose complications such as caries, periodontal disease, root resorptions, and loss of patient cooperation [1]. Shortening the duration of treatment can prevent or reduce the severity of these problems, but it is challenging for orthodontists since it requires acceleration of tooth movement.

Orthodontic tooth movement (OTM) is composed of a series of inflammatory-like reactions [2]. Any intervention that

affects these reactions could change the rate of tooth movement. Many studies have tried to find a method to accelerate tooth movement by modifying cellular response to mechanical forces without traumatizing teeth or periodontium such as administration of osteocalcin [3], nitric oxide [4], the active form of vitamin D3 (1, 25$(OH)_2$D3) [5], prostaglandin E2 [6], or other physiologic agents. Despite the effectiveness of these substances in accelerating OTM, some of them impose systemic effects on body metabolism and cause local pain following alveolar bone injections, which limit their application in orthodontic treatment [7].

Recently, low-level laser irradiation (LLLI) has been shown to have biological effects on hard and soft tissues in dentistry. LLLI is a simple, non-invasive, and effective way to reduce post-adjustment pain [8], the pain during OTM [9] and also the pain associated with the placement of orthodontic elastomeric separators [10–12]. Several studies have shown the efficacy of LLLI with a wavelength between 800 and 980 nm, in pain reduction after dental treatment [8–10, 12, 13]. LLLI can also be used in the treatment of traumatic ulcers in the oral mucosa caused by the appliance and bone regeneration in sutures [14]. Some animal and human studies have showed LLLI increased the rate of OTM or alveolar bone remodeling rate, without harming the teeth [7, 9, 14, 15]. Some studies, however, reported controversial results regarding the effect of LLLI and failed to show any significant difference between the rate of OTM on the laser side and the non-irradiated side [1, 16, 17].

LLLI stimulates bone remodeling by induction of osteoblasts proliferation in the tension side and osteoclasts in the pressure side and also by stimulating collagen synthesis [14]. On the other hand, some researchers believe that the inhibitory effects of lasers on prostaglandins prevent tooth movement [18].

OTM is based on forced-induced periodontal ligament (PDL) and alveolar bone remodeling [19]. Mechanical stimuli exerted on a tooth cause an inflammatory response in the surrounding periodontal tissues [19]. Inflammatory mediators, such as cytokines, that are released in this process are responsible for the apposition-resorption process of the bone [19]. They are key mediators of tissue damage, tooth movement, normal bone turnover, and remodeling [19, 20]. Most cytokines are released in response to orthodontic mechanical stress on periodontium cells [21]. Cytokines are classified as either pro-inflammatory or anti-inflammatory [22]. Pro-inflammatory cytokines include tumor necrosis factor, interleukin1 (IL-1), IL-2, IL-6, and IL-8. Anti-inflammatory cytokines are IL-4, IL-10, and IL-13 [20].

According to different studies, one method of evaluating the biologic events of the underlying PDL is assessing the cytokines in gingival crevicular fluid (GCF) [20, 21]. It has been shown that the levels of IL-6 are elevated in GCF during orthodontic treatment, and this increase plays an important role in the bone resorption-apposition process [20, 21]. Few human studies have evaluated the long-term changes of cytokine levels during orthodontic treatment [20, 23]. Since the magnitude of force varies throughout the stages of orthodontic treatment, the levels of cytokines may also be different at each stage.

There are some studies about the effect of LLLI on the rate of OTM with a wavelength between 780 and 850 nm [7, 9, 14, 15, 18, 24], but there is no study about the effect of 980 nm continuous wavelength diode laser. Also, only one study investigated the biologic changes in GCF during LLLI [15]. Therefore, the aims of this study were to determine the effects of a 980-nm diode gallium-aluminum-arsenide (GaAlAs) laser on the velocity of OTM and on IL-6 levels during various stages of orthodontic treatment.

### The null hypothesis

The null hypothesis was that the 980 nm diode laser increased the rate of OTM and IL-6 levels in GCF during canine retraction.

## Materials and methods

Ethical approval was sought and granted by the by the medical ethical committee and the research vice chancellor of the Shahid Sadoughi University of Medical Science for this randomized double-blind clinical trial. In this investigation, 11 female patients, aged 14 to 25 years (mean 19±4.21 year), attending the Department of Orthodontics, Shahid Sadoughi School of Dentistry in Yazd, Iran, were selected to participate in this study. This sample size was chosen based on the previous studies [1, 7, 24, 25].

The criteria for selecting patients was as follows: (1) a diagnosis of bimaxillary dentoalveolar protrusion which needed four first premolar extraction and canine retraction, (2) no systemic disease,(3) no previous extraction of permanent teeth, (4) not receiving medical treatment that could interfere with bone metabolism, such as NSAIDS or doxycycline, (5) a good level of oral hygiene, having no inflammation and obvious calculus and the bleeding on probing and plaque index less than 15 %, (6) no periodontal disease or radiographic evidence of bone loss, (7) no pregnancy during treatment, and (8) agreement to sign the informed consent by the patient or their parents. Exclusion criteria included patient unwillingness for participation in the study, inappropriate oral hygiene, prolonged drug consumption during treatment, and repeated bracket debonds.

Diagnosis and treatment planning were based on standard records, including photographs, study models, lateral cephalograms, and panoramic radiographs.

### Orthodontic treatment

All patients had bimaxillary dentoalveolar protrusion with a convex profile and class I canine and molar relations. Before beginning the treatment, oral hygiene instruction was given to the patients. During the course of the treatment, oral hygiene instructions were repeated, and the patients were instructed to use chlorhexidine gluconate 0.2 % mouth rinse for a short time, if needed.

A preadjusted 0.022 × 0.028″ edgewise appliance (GAC International, Bohemia, NY) was used for bonding, and a transpalatal bar (0.032″ SS wire) [7, 24] was used for posterior anchorage. The treatment began on the same day for all patients, and leveling and aligning were completed in 6 months. Three weeks following the extraction of the first premolars of each jaw, canine retraction and laser irradiation began.

The maxillary canines were separately retracted using NiTi closed coil springs (GAC International, Bohemia, NY) on rectangular SS wires. The total force used was 150 g, which was measured by a special gauge (Dentaurum, Ispringen, Germany). The first and second molars and the second premolar on each maxillary side were ligated by figure 8 SS wire (0.012″) to reinforce the anchorage. Spring reactivation was made on the 21st of each month until the canine retraction was completed.

### Laser irradiation

The study was carried out in a split mouth design to limit the confounding factors. A diode laser (GaAlAs) (A.R.C. Laser GmbH, Nürnberg, Germany) emitting infrared radiation at 980 nm, with an output power of 100 mW, dose of 5.6 J/cm$^2$ operating in a continuous-wave mode was used. The laser irradiation was only performed on the maxillary arch. All irradiations were performed by the one operator in one maxillary quadrant, which was randomly selected, as laser group (LG). Random selection was done by one operator; the second clinician responsible for the treatment stages was unaware of the side chosen for laser irradiation. The tip of the laser was held perpendicular, and in contact with the mucosa during the irradiation. A total of six irradiations each time, three from the buccal side and three from the palatal side, were carried out to cover the periodontal fibers and the alveolar process around the canine teeth. The irradiation time for the cervical and middle third of the tooth was 10 s, and 8 s for the apical third of the tooth. The laser irradiation was applied on days 0, 7, 14, 21, and 28 every month during canine retraction phase [15]. The laser was turned off and placed on the other quadrant for the pseudo-irradiation as a control group (CG), so that the patients were unaware of the irradiated side.

### Assessment of the amount of canine retraction

Dental casts were made at the beginning of the trial, the end of the aligning and leveling phase, on the 21st day of each month during canine retraction, and at the end of this phase. The amount of canine movement in millimeter was measured using the distance from the tip of the canine cusp to the tip of the mesiobuccal cusp of the first molar [7, 9] by means of a digital caliper (Pittsburgh, PA), and this amount was recorded as "Distance."

The velocity of the movement was obtained from the following formulation: $V = d/t$, where $V$ is the velocity of canine movement, $d$ is the amount of canine movement in millimeter at the end of canine retraction phase, and $t$ is the duration of the treatment [9].

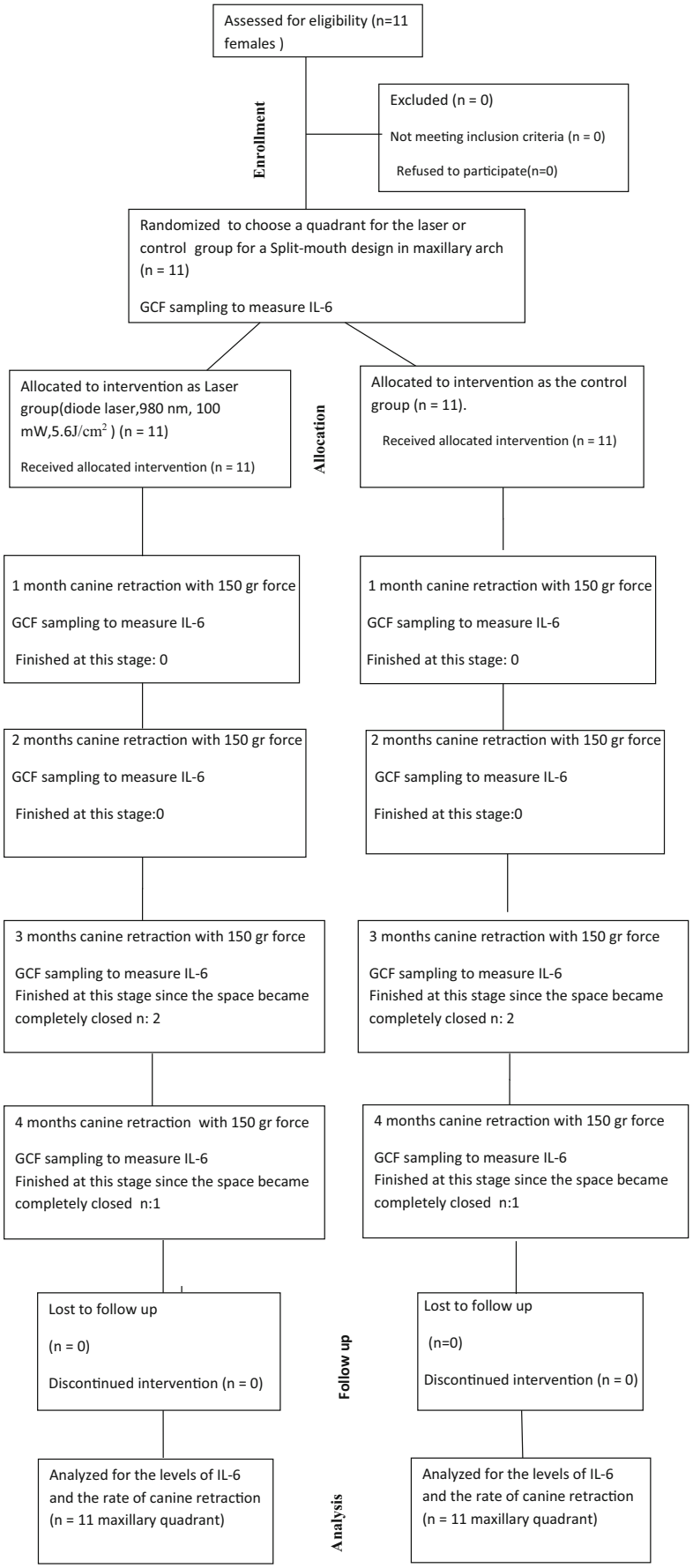
### GCF sampling

GCF samples were collected at baseline, and before placing the wire for alignment (stage 0) to measure IL-6 concentration. The second sample was taken at the end of alignment and leveling phase (stage 1). During the canine retraction, GCF was obtained on the 21st day of each month before laser irradiation and reactivation of the appliance (stage 2–5). The 21st day was selected because spring reactivation was made on this day each month during canine retraction. The GCF sampling was only done in the maxillary arch. In two patients, the canine retraction finished at the end of stage 3, and another patient was finished in stage 4. A CONSORT diagram showing the flow of the patients through each stage of the trial is shown in Fig. 1.

GCF samples were collected using high-purity filter papers used as paper strips (Munketell Filter AB, Falun, Sweden,). The 10 × 2 mm paper strips were carefully weighted by digital scale, and each strip was 0.0017 g. The paper strip was inserted into the distal site of crevice [20] until a mild resistance was felt and left there for 60 s. To minimize the risk of contamination of GCF samples, supragingival plaque was carefully removed without traumatizing the gingival tissues.

Following isolation of the sample sites with cotton rolls, the plaque was removed and the surface of the tooth was air-dried for 15 s. Samples contaminated with blood or saliva were excluded from the study. Figure 2 depicts the collection method of the GCF samples.

Immediately after GCF collection, the strips were transferred to 1.5-ml sterile tubes that contained 250-μl phosphate-buffered saline. Parafilm® (Bemis; Neenah, Wiscons in, USA) was used to seal the tubes. The tubes were stored at −20 °C until the start of the experiment and then sent to the Immunogenetics Department of the Bu-Ali Research Institute. An immunoassay kit (eBioscience, Ltd., Ireland, UK) was used to measure the IL-6 concentration in each sample in


Assessed for eligibility (n=11 females )
Enrollment
Excluded (n = 0)
Not meeting inclusion criteria (n = 0)
Refused to participate(n=0)
Randomized to choose a quadrant for the laser or control group for a Split-mouth design in maxillary arch (n = 11)
GCF sampling to measure IL-6
Allocated to intervention as Laser group(diode laser,980 nm, 100 mW,5.6J/cm2 ) (n = 11)
Received allocated intervention (n = 11)
Allocation
Allocated to intervention as the control group (n = 11).
Received allocated intervention (n = 11)
1 month canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage: 0
1 month canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage: 0
2 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage:0
2 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage:0
3 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage since the space became completely closed n: 2
3 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage since the space became completely closed n: 2
4 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage since the space became completely closed n:1
4 months canine retraction with 150 gr force
GCF sampling to measure IL-6
Finished at this stage since the space became completely closed n:1
Lost to follow up
(n = 0)
Discontinued intervention (n = 0)
Follow up
Lost to follow up
(n=0)
Discontinued intervention (n = 0)
Analyzed for the levels of IL-6 and the rate of canine retraction (n = 11 maxillary quadrant)
Analysis
Analyzed for the levels of IL-6 and the rate of canine retraction (n = 11 maxillary quadrant)

◀ **Fig. 1** CONSORT flow diagram showing progress of subjects through trial

picograms per milliliter (pg/mL). This study was done in 11 months.

### Statistical analysis

Statistical tests were done using PASW® version 18 (SPSS; Chicago, IL, USA). One sample Kolmogorov-Smirnov (K-S) test was used to evaluate the normal distribution of the data; Wilcoxon signed-rank test was used to compare the mean rank of the data with abnormal distribution. General linear model (GLM) repeated measures test was used to compare the IL-6 concentration and the distance variable between the different treatment stages (0–5). Bonferroni paired comparison was used to compare the pairs of different treatment stages. A paired *t* test was used to compare the IL-6 concentration between LG and CG. Pearson and Spearman correlations were used to evaluate the relationship between the movement extent and the changes in IL-6 levels. The Spearman nonparametric test was also used to calculate the correlation coefficient for the velocity of OTM. *P* value of less than 0.05 was defined to be statistically significant for all the tests.

## Results

The K-S test showed that the distance variable was normally distributed in both groups ($P > 0.05$). Table 1 shows the mean and the standard deviation (SD) of the distance variable in both LG and CG in different stages. Bonferroni paired comparisons revealed no significant difference in distance between stages 0 and 1 and also between stages 0 and 2 ($P > 0.05$). Movement velocity did not have a normal distribution ($P < 0.05$). The correlation coefficient for the velocity in both sides was 1.00. Although the mean value of retraction velocity was higher in the LG and the *P* value approached 0.05 (0.068), the results failed to show a significant difference between the mean rank of canine velocity in both groups. Figure 3 shows the mean retraction velocity of canine tooth in both groups.

**Fig. 2** The collection of gingival crevicular sample

**Table 1** The mean and standard deviation of distance parameter in the canine retraction phase in laser and control groups at different stages

| | | *N* | Mean ± std. deviation |
|---|---|---|---|
| Laser group | Stage 0 | 11 | 2.18 ± .15 |
| | Stage 1 | 11 | 2.22 ± .12 |
| | Stage 2 | 11 | 1.98 ± .19 |
| | Stage 3 | 11 | 1.77 ± .31 |
| | Stage 4 | 9 | 1.68 ± .20 |
| | Stage 5 | 8 | 1.57 ± .14 |
| Control group | Stage 0 | 11 | 2.15 ± .13 |
| | Stage 1 | 11 | 2.21 ± .09 |
| | Stage 2 | 11 | 1.99 ± .17 |
| | Stage 3 | 11 | 1.82 ± .27 |
| | Stage 4 | 9 | 1.73 ± .17 |
| | Stage 5 | 8 | 1.59 ± .15 |

Stage 0: before the treatment (baseline). Stage 1: the end of leveling and aligning phase. Stage 2: the end of first month of canine retraction. Stage 3: the end of second month of canine retraction. Stage 4: the end of third month of canine retraction. Stage 5: the end of fourth month of canine retraction

IL-6 concentrations showed a normal distribution. Table 2 shows the mean and SD of IL-6 levels in both groups. There were significant differences in IL-6 levels between stages 0 and 5 and also between stages 1 and 5 in LG ($P < 0.05$); none of the paired comparisons in the CG group showed a significant difference. The results also showed that no significant differences were present between the groups regarding IL-6 levels at different stages of treatment ($P > 0.05$).In addition, no correlations existed between the IL-6 levels and canine movement in different stages ($P > 0.05$).

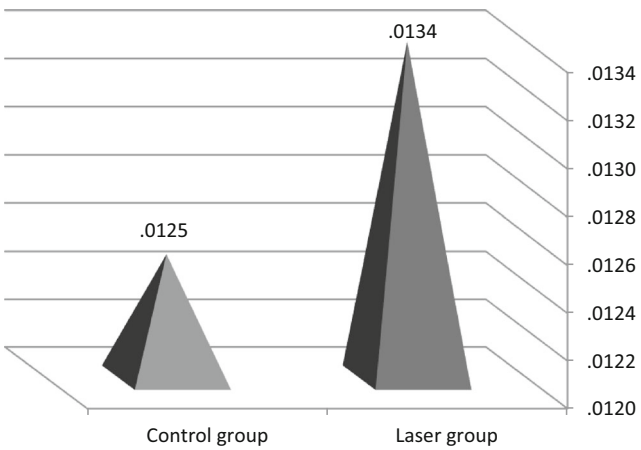



**Fig. 3** Mean retraction velocity of the canine tooth of laser and control groups

**Table 2** The mean and standard deviation of IL-6 concentration in laser and control groups in different stages (pg/mL)

| | | *N* | Mean ± std. deviation |
|---|---|---|---|
| Laser group | Stage 0 | 11 | .06 ± .01 |
| | Stage 1 | 11 | .22 ± .19 |
| | Stage 2 | 11 | .45 ± .31 |
| | Stage 3 | 11 | .48 ± .31 |
| | Stage 4 | 9 | .5 ± .32 |
| | Stage 5 | 8 | .76 ± .44 |
| Control group | Stage 0 | 11 | .06 ± 01 |
| | Stage 1 | 11 | .12 ± .54 |
| | Stage 2 | 11 | .49 ± .40 |
| | Stage 3 | 11 | .33 ± .26 |
| | Stage 4 | 9 | .5 ± .33 |
| | Stage 5 | 8 | .48 ± .30 |

Stage 0: before the treatment (baseline). Stage 1: the end of leveling and aligning phase. Stage 2: the end of first month of canine retraction. Stage 3: the end of second month of canine retraction. Stage 4: the end of third month of canine retraction. Stage 5: the end of fourth month of canine retraction

## Discussion

Laser irradiation has a variety of effects on tissues, ranging from biostimulation to photo disruption [14]. The extent of these effects depends on the wavelength, total dose, frequency, and laser irradiation time [14, 26].

Some studies have reviewed various applications of the biostimulation effect, which include enhancement of bone regeneration in midpalatal sutures after maxillary expansion, reduction of posttreatment pain after application of orthodontic force, and OTM acceleration [7, 9, 15, 26].

Because hemoglobin and water show a low level of absorption of infrared radiation, penetration depth is high in the irradiated tissues [14]. The infrared laser was selected for this study because our objective was to stimulate bone cells.

In a study in animals, Luger et al. [27] used a 64 J/cm$^2$ dose over a period of 14 days. This was quite high within the focused area, but the authors believed that scattering reduced the energy level of the laser to between 3 and 6 % of the original intensity while it was being transmitted through the tissues. This study reported a positive effect of LLLI on bone repair [27]. In our study, we used a lower dose (5.6 J/cm$^2$) at six different points around the canine tooth on the buccal and palatal sides. This protocol could result in a more homogeneous distribution of the energy of the laser than in the study of Luger et al. [27].

Sousa et al. [28] conducted a systematic review and declared that the most effective energy input for a single tooth retraction was 2 to 8 J per tooth at a frequency of application of 1 to 5 days per month for 3 to 6 months. Therefore, we used the 5.6 J/cm$^2$ dose at five applications per month.

The present study used a continuous wave GaAlAs laser (980 nm) at 100 mW with a dose of 5.6 J/cm$^2$. The difference between the distance mean at various stages of treatment was significant; the mean retraction velocity in the LG showed better results as well, with a $P$ value trending toward significance ($P = 0.068$).

The results of our study are compatible with the findings of a study conducted by Kansal et al. [24]. These researchers used the diode laser in 10 patients and reported that the rate of tooth movement in the LG was better than in the CG, but the results were statistically insignificant. The laser parameters of their study were 904 nm, 12 mW, 4.2 J/cm$^2$. A total of 10 irradiations (10 s at each point) were used each time in the LG; this laser frequency was similar to that of our study.

In another study, Hosseini et al. [25] used the 890 nm diode laser and reported that the energy dose used (72 J per tooth) did not significantly increase dental movement, in spite of the mean canine retraction being greater on the side where the laser was used. The failure to obtain a positive result in their study could be due to the pulsed-mode application of the laser, the inadequate frequency of laser irradiation (2 weeks with 48 h intervals), or the high dose used in each application.

A recent systematic review showed that LLLI is dose-dependent and reported that LLLI has shown some degree of efficacy with the correct dosage [28]. Another systematic review about surgical and non-surgical interventions on accelerating OTM revealed that the clinical efficacy of LLLI was not significant [17].

The study of Limpanichkul et al. [1] using a diode laser (860 nm) with an energy density of 25 J/cm$^2$ at the surface level found no increase in tooth movement in the LG. They stated that the amount of absorbed energy by the tissues had an effect on the tissue response to LLLI.

Other studies have reported a significant positive stimulatory effect of LLLI on the rate of tooth movement, which may be due to the difference in the total dose and the wavelength of the diode laser. With regard to the energy and wavelength of the GaAlAs laser apparatus, the wavelengths used by Youssef et al. (809 nm) [9], Cruz et al. (780 nm) [7], Genc et al. (808 nm) [15], and Yoshida et al. (810 nm) [29] were shorter than those in our study. Furthermore, the energy densities used by Youssef et al. (8 J/cm$^2$) [9], Cruz et al. (50 J/cm$^2$) [7], Genc et al. (0.71 J/cm$^2$) [15], and Yoshida et al. (54 J/cm$^2$) [29] differed from those in the current study.

Altan et al. [14] stated that the most effective wavelength for LLLI is between 550 and 950 nm. Takeda [30] reported the existence of significant biostimulation effects of LLLI (904 nm, 20 J/cm$^2$) on bone metabolism in rats. He indicated that these effects occurred at this dosage (approximately 900 nm, 20 J/cm$^2$); in keeping with their

findings, other studies have indicated that lower dosages showed non-significant results [30, 31].

Saito and Shimizu [26] studied the effect of LLLI on rapid midpalatal suture expansion in rats and compared the bone regeneration obtained with and without laser irradiation. They found that bone regeneration was stimulated by the consecutive application of the laser in the first days after midpalatal expansion. Their LG showed 20 to 40 % better results than did their CG.

In the present study, the mean canine retraction velocity was better in the LG, and there was definitely a tendency for more canine retraction in the LLLI. However, the study did not provide strong evidence about the efficacy of laser treatment, possibly due to the low sample size. The difference in mean velocity between the two groups may have become statistically significant with a larger sample size.

When PDL cells are subjected to orthodontic forces, some substances are released from these cells [20]. These substances play a role in maintenance and remodeling of the PDL and the adjacent alveolar bone metabolism [20, 32]. The detection of these substances is possible through the GCF.

GCF is an osmotically mediated exudates present in the gingival sulcus and is primarily consisted of serum [33]. Its composition changes in the presence of bacteria and gingival inflammation [33]. In general, the GCF components are microbial dental plaque and its toxin, imonoglubiline, host inflammatory cells, host tissue, lysosomal enzymes, and serum [34].

GCF analysis is a simple and non-invasive method that allows repetitive sampling from the same site of the gingival sulcus [35]. In recent years by identifying different biologic markers in GCF, biological aspects of OTM in PDL have been assessed [20, 32, 36]. According to some studies, the GCF composition changes during orthodontic treatment [36], and the GCF volume increases during OTM [20, 23, 37].

IL-6 is a multi-functional cytokine that is produced by a variety of cells. Depending on the nature of the target cells, it acts on a wide range of tissues, facilitating growth-induction, growth-inhibition, and differentiation. IL-6 also regulates immune responses (including the acute phase of the inflammatory response), assists in hematopoiesis, and may also play a role in host defense mechanisms [38, 39].

Inflammatory cytokines such as IL-6 are important for stimulating the formation of osteoclast precursors, osteoclast proliferation, and bone-resorbing activities of the osteoclast post-procedure. More specifically, they regulate the regional remodeling activity of the bone and the acute inflammatory response at the beginning of orthodontic treatment [21, 40].

Most of the studies evaluated the cytokine levels in GCF for relatively short periods of time [33, 36]. Because the magnitude of force is much lower in the alignment and leveling phase of orthodontic treatment than in the distalization phase, there might be different levels of cytokines in these stages. Therefore, the long-term changes of the IL-6 concentration in the GCF at different stages of orthodontic treatment were the subject of the present study.

Since it has already been demonstrated that the IL-6 concentration does not differ between the tension and compression sites [41], the present study was conducted only on the compression (distal) site of the canine to achieve a fixed set of input variables for the sake of avoiding ambiguity. Furthermore, to eliminate the probable difference between the two sexes in enzymatic activity, only females were included in the study.

The results of this study showed that the increase in the volume of IL-6 on day 21 of each month during canine distalization was not significant. These results are similar to those of the research conducted by Basaran et al. [20]. They performed a long-term study to determine the levels of IL-2, 6, and 8, concluding that the levels of IL-6 did not increase significantly at the end of each month during canine retraction.

In the present study, the levels of IL-6 increased from stage 0 to 1 in LG, but this was not statistically significant. This result was in contrast to the study conducted by Basaran et al. [20], who reported that the concentration of the interleukins increased significantly after aligning and leveling the teeth.

Van Gastel et al. [23] in their study stated that the increased gingival inflammation during orthodontic treatment might lead to increased GCF production and consequently the dilution of the cytokines. The non-significant increase in the IL-6 levels from stage 0 to 1 in our study might be related to the dilution of the cytokines.

The results of the present study showed that there was a significant difference in the levels of IL-6 between stage 1(at the end of the alignment and leveling phase) and stage 5 in LG. This could be due to the effect of LLLI in slightly increasing the rate of OTM. No other study has investigated the effect of LLLI on the IL-6 levels yet.

Furthermore, the statistical analysis of the present study revealed a significant difference between the levels of IL-6 between the baseline and the final stage of the distalization phase in the LG, but there was no significant difference between any pair of stages in the CG. The results also showed that there was no significant difference between the LG and CG with respect to the IL-6 levels at different stages of treatment ($P > 0.05$). In the present study, the canine retraction was performed in both groups, and the only difference between these groups was the laser irradiation in LG. LLLI had only slight effects on increasing the velocity of tooth movement in this group, and the non-significant difference between these two groups in IL-6 levels might be the result of simultaneous canine retraction on both sides.

In a study conducted by Uematsu et al. [21], the levels of different pro-inflammatory cytokines such as TNF-α, EGF, and IL-6 were evaluated, and it was shown that the levels of

these cytokines increased significantly during the course of orthodontic treatment. The researchers stated that the IL-6 concentration was significantly higher at 24 h after applying orthodontic force. In a similar study, Davidovitch et al. [42] reported a significant increase in the mean concentration of cytokines during the early days of orthodontic treatment and a decrease after 7–10 days. It was declared that this initial increase as an inflammatory response was important for orthodontic force-induced bone remodeling.

Along the same lines, Ren et al. [22] recently reported a significant elevation in the levels of pro-inflammatory cytokines such as IL-6 and IL-1 β after 24 h of force application in the early stage of tooth movement. All cytokine levels were reduced to their baselines at different time points during the linear stage of OTM.

They also reported that there was no further increase after 2–3 months of orthodontic treatment.

In the present study, the maximum mean concentration of IL-6 in the LG was in stage 5. The difference between the results of the present study and the abovementioned studies could have resulted from the different times of GCF sampling. In this study, only long-term evaluation of the IL-6 concentration was conducted. The GCF samples were taken at baseline (stage 0), stage 1, and on day 21 of each month during canine retraction (stages 2–5). The increase in the mean concentration of IL-6 between stage 0 and 1 was not significant. This is because the second GCF collection (stage 1) was performed 6 months after stage 0. Other studies [21, 33, 36] have evaluated the increase in the cytokine levels during a shorter period of 24 h or 1 week, which could explain the difference in the results.

According to the conflicting results related to the efficacy of LLLI on the rate of tooth movement, further studies with a larger sample size including female and male subjects are suggested in order to determine the most efficient diode laser wavelength to accelerate the velocity of tooth movement. As a result, the levels of different cytokines could be considered more precisely. Furthermore, additional studies are needed to determine cytokine levels during different stages of orthodontic treatment, such as at the finishing stage and at both relatively short (24 h) and longer periods of time.

## Conclusion

The diode laser (980 nm) with adjusted parameters used in this study slightly enhanced the rate of OTM. Distalization of the teeth caused a significant increase in the concentration of IL-6, but the increase in the mean concentration of IL-6 was not statistically significant between the two sides. Solid evidence could not be provided to support the efficacy of a diode laser in accelerating OTM.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Ethical standards** This clinical trial was verified by the ethical committee and the research vice chancellor of Shahid Sadoughi University of Medical Science by the P/17/1/31871 ethical code. All the patients or their parents signed informed consent.

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