

# The Role of Local Injection of Tnf- $\alpha$ Antagonist on Orthodontic Tooth Movement in Rabbits

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## ABSTRACT

**INTRODUCTION** Orthodontic tooth movement supposed to be mediated by several host mediators such as interleukin-1 and tumor necrosis factor- $\alpha$ . The aim of this study was to investigate the effect of infliximab (tumor necrosis factor- $\alpha$  antagonist) local injection on orthodontic tooth movement.

**Methods:** orthodontic appliance was placed on the upper central incisors of 22 adult male local bred rabbits for 18 days to create a space between the two central incisors. The rabbits were divided into two equal groups, the experimental group received 5mg/kg subperiosteal injection of tumor necrosis factor-alpha antagonist (infliximab) in the labial side of the upper central incisor in three time intervals (0,7,13 days) of the study period. The control group received equivalent volume of normal saline in the same location as in experimental group. Clinical measurement of the space between the central incisors were done in (5, 9, 13, 18) days.

**RESULTS** There was a significantly smaller rate of tooth movement in experimental group than control group represented by smaller spaces created.

**Conclusions:** local injection of infliximab decreases the movement of the tooth during orthodontic treatment.

**Keywords:** infliximab, TNF- $\alpha$ , Orthodontics, tooth movement

## INTRODUCTION

The purpose of orthodontic treatment is to move teeth as efficiently as possible with minimal adverse effects for the teeth and supporting tissue. During orthodontic tooth movement, bone resorption can be initiated at the periodontal surface of the alveolar bone.<sup>(1)</sup>

Many attempts have been done to decrease bone resorption during orthodontic treatment using different systemic and local application of medications and the intake of dietary supplements, such as minerals<sup>(2)</sup>, hormones<sup>(3)</sup>, proteins<sup>(4)</sup> and immunomodulators<sup>(5)</sup>. Gamberio *et al* 2008<sup>(6)</sup> used celecoxib, a nonsteroidal anti-inflammatory drug (NSAID), as an intra pre-torial injection (10 mg/kg) in wistar rats with orthodontic moved teeth. The amount of tooth movement was significantly reduced in rats submitted to short and long term celecoxib administration. Decreased tooth movement was also recorded by Liu *et al* 2004<sup>(7)</sup> after local injection of Clodronate solution in subperiosteal area adjacent to the upper molar of rats in a dose of 2.5, 10 and 40 mM. The same results was obtained by Venkataramana *et al* 2012<sup>(8)</sup> when they examined the influence of local administration of bisphosphonate (pamidronate) as an anti-resorptive drug, and by Seifi *et al* 2004<sup>(9)</sup> who studied the effect of Aspirin (NSAIDs) 100 mg/kg in one dose per day on the rate of orthodontic tooth movement in albino Newzealand rabbits. When the orthodontic appliance was inserted between 1st molar and incisors of the mandible, there was a significant decrease in the rate of tooth movement. Diclofenac have been shown to inhibit ortho-

odontic tooth movement totally in a wistar male rat (10 mg/kg at days 1 and 3), in which mesial tipping of the first molars by coil spring was induced by a force of 50 and 100 cN.<sup>(10)</sup>

The orthodontic loading leads to a focal tissue injury and consequently, an aseptic inflammatory response characterised by the release of several important inflammatory mediators on periodontal tissues such as the cytokine.<sup>(11)</sup>

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a potent immunomediator and proinflammatory cytokine that is rapidly upregulated in the brain after injury and secreted by macrophage, monocyte, and neutrophile<sup>(12)</sup>. Tumor necrosis factor-alpha functions as a pro-inflammatory mediator contributing to the development of such diseases as rheumatoid arthritis<sup>(13)</sup>, multiple sclerosis<sup>(14)</sup> and periodontitis<sup>(15)</sup>.

The pro-inflammatory cytokines, including TNF- $\alpha$ , are thought to play a role in bone remodeling and osteoclast differentiation. In rats and in humans, TNF- $\alpha$  modifying processes directly associated with tooth movement, and it may also induce mediators of the inflammatory process, which will then influence osteoclast recruitment and function. Thus orthodontic tooth movement increases the levels of TNF- $\alpha$  in the periodontal tissues<sup>(16)</sup>

Bletsa *et al* 2006<sup>(17)</sup> showed that increased expression of IL-1  $\alpha$  and TNF- $\alpha$  occurred as early as one day after mechanical force application at both compression and tension areas.

Tooth movement experiments in rats showed that

excessive orthodontic force induced the expression of TNF- $\alpha$  in periodontal tissues. In addition, orthodontic tooth movement increases the levels of TNF- $\alpha$  in the gingival sulcus in humans, suggesting an important role for TNF- $\alpha$  in orthodontic tooth movement.<sup>(18)</sup>

TNF- $\alpha$  antagonists block TNF- $\alpha$  released by activated monocytes, macrophages, and T-lymphocytes, which are essential for inflammatory responses following force application<sup>(19)</sup>.

One type of TNF- $\alpha$  antagonists is Infliximab and its use has now expanded to inflammatory bowel disease, ankylosing spondylitis, plaque psoriasis, rheumatoid arthritis, and Crohn's Disease<sup>(20)</sup>.

A study was done on *Macaca fascicularis* monkey by Assuma *et al* 1998<sup>(21)</sup> with induced periodontitis and alveolar bone loss through tying porphyromonas gingivalis-soaked suture ligatures around the posterior mandibular teeth. The animals received local injection of soluble receptors to IL-1 and TNF to sites of induced periodontal destruction three times each week for 6 week. The results indicate inhibition of the inflammatory cells in close proximity to bone by approximately 80% and the formation of osteoclasts was reduced by 67% at the experimental sites compared with that at the control sites, and the amount of bone loss was reduced by 60%.

In an attempt to decrease the proinflammatory effect of TNF- $\alpha$  on orthodontic tooth movement, this study was done to investigate the role of local inflix-

imab (TNF- $\alpha$  antagonist) injection on orthodontically moved teeth in rabbits.

## METHOD

This study used 22 male local bred rabbits weighing 1.5-2 kg. At the beginning of the experiment, all rabbits were kept under standardized laboratory conditions of light-and-dark schedule and relative humidity for 7days, fed ad libitum with commercial pellets and water from thick-walled glass dishes. At day of orthodontic appliance insertion, the rabbits were anaesthetized by intramuscular injections of xylazine 2%(4mg/kg B.W.)and Ketamine hydrochloride (40mg/kg B.W)mixture<sup>(22)</sup>, and orthodontic appliances were placed for all rabbits for 18 days. The orthodontic appliance consisted of two mini buccal tubes bonded to the labial surface of the upper central incisors vertically positioned parallel to long axis of the incisors in the cervical third of clinical crown. L-shaped wire consist of 2 pieces of 0.017\*0.025 inch stainless steel wires, was inserted in the both tubes. The force was applied by light strength nickel-titanium open coil spring which was fitted over the two horizontal wires sections between the two upper central incisors(fig.1 and 2). The force level after activation was approximately 35gm measured using Bolye gauge. This force resulted in distal movement of each upper central incisors gaining median space between the two central incisors.

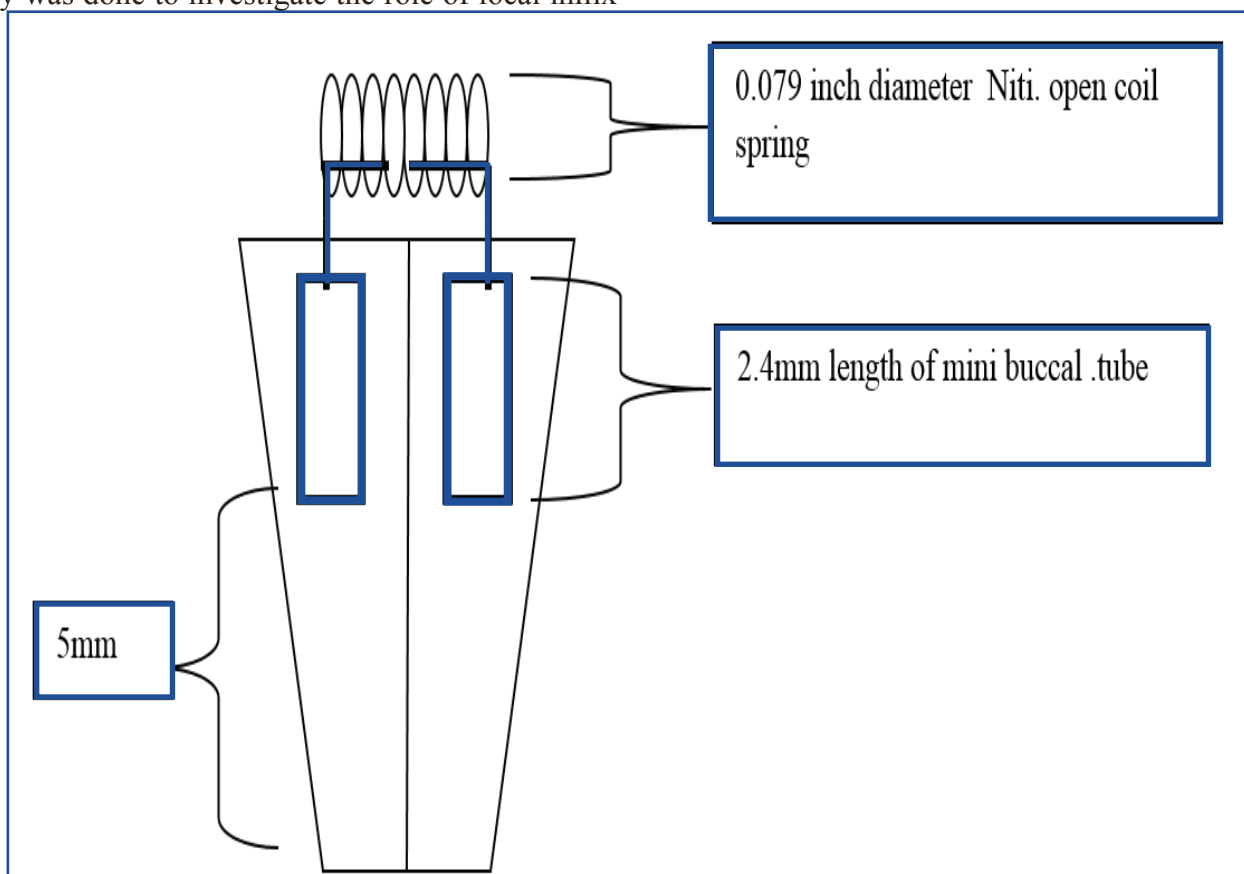


Figure 1: diagram showing the orthodontic appliance used in the study

The rabbits were divided equally into 2 groups (11 rabbit for each): an experimental group which received subperiosteal injection of 0.1 ml of TNF- $\alpha$  antagonist (infiximab 5 mg/ kg) in the labial side cervically<sup>(23)</sup> (fig.3). The remaining 11 rabbits were considered as control group which received 0.1ml normal saline injection in the same site as experimental group. The local injection of both infiximab and normal saline was given in three different time

intervals (1st, 7th and 13th day) following insertion of the appliance. To quantify and record the amount of tooth movement, four measurements were performed for each rabbit by measuring the distance between the two mid-point of the mesial surface at cervical line of central incisors at (5th, 9th, 13th, 18th day) using digital vernier sensitive to 0.01 mm. This point was chosen in order to stick to a fixed point during measurement.



Figure.2:orthodontic appliance on upper central incisors before activation



Figure 3: infiximab injection site.

statistics: means and standard deviations were calculated for both groups. Unpaired student t-test, showing differences in means of spaces between control group and experimental group at different times. Measurements were in mm,(P value $\leq$  0 .05 was considered statistically significant).

## RESULTS

The insertion of the designed orthodontic appliance resulted in distal movement of the two central incisors with minimum distortion for the gingival tissue and for the appliance in both experimental and control group. (fig.4)



Figure 4:Orthodontic appliance on upper central incisors at the end of the study.

The injection of infiximab in the labial side of experimental group resulted in an obvious differences from the control group which were recorded and

measured clinically. The statistical analysis of the clinical measurement in day(5,13,18) revealed a significant difference between experimental and control

groups ( $P < 0.001$ ). There was highly significant difference in mean of the space between the two groups in day 5, where the mean space was ( $2.391 \pm 0.406$  mm) in experimental group, while in control group it was ( $3.373 \pm 0.361$  mm). On the other hand, there was no significant difference between experimental and control group at day 9, where the mean of space in the experimental group was ( $3.482 \pm 0.435$ ), while

in control group was ( $3.664 \pm 0.520$ ). In day 13, the mean of space was ( $4.046 \pm 0.463$  mm) in experimental (infiximab given) group, while in control group it was ( $5.055 \pm 0.423$  mm). In day 18 the mean space was ( $4.400 \pm 0.322$  mm) in experimental group, while in control group it was ( $5.491 \pm 0.356$  mm). as shown in (figure:5)

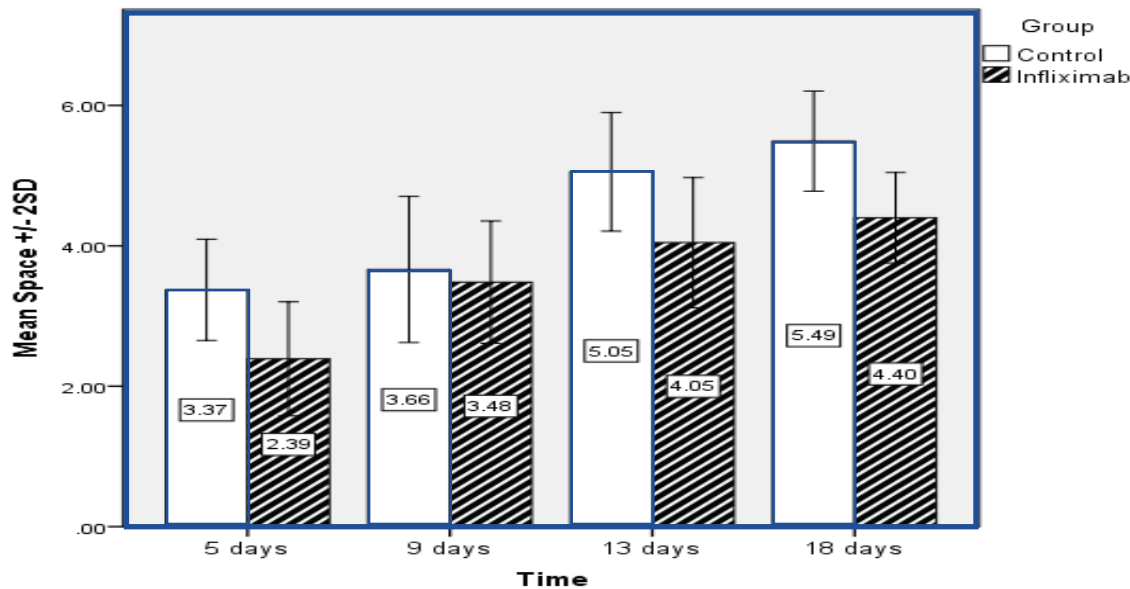


Figure 5: Unpaired student t-test of means and standered deviation( $\pm S$ ) of spaces between controls and infiximab(experimental) group.

## DISCUSSION

Orthodontic tooth movement occurs by the remodeling of alveolar bone as a result of the force that is exerted on the periodontium. In this study, a non-human primate model was used to assess the effect of local administration of TNF- $\alpha$  antagonist (infiximab) on the rate of tooth movement in an attempt to interfere with cytokines which are usually elevated during orthodontic tooth movement<sup>(17)</sup>. Up to our knowledge there is no previous clinical study on the effect of local infiximab injection on orthodontic tooth movement. The results of this study are comparable with those obtained by Andrade *et al*, 2007<sup>(16)</sup> in a nonhuman primate model which use the orthodontic appliance of NiTi coil spring between the maxillary right first molar and the incisors in Wild-type mice (WT) and p55-or TNF-RI deficient mice (p55-/-). The levels of TNF- $\alpha$  and chemokines were evaluated in the periodontal tissues with a significantly smaller rate of tooth movement, and lower number of TRAP-positive osteoclasts in p55-/- mice than that observed in Wild type mice.

The obtained results of this study is similar to the results obtained by Liu *et al* 2004<sup>(7)</sup> who injected Clo-

dronate into the sub-periosteum area adjacent to orthodontically moved tooth. The clodronate injection caused a significant and dose dependent reduction in tooth movement in the rats.

The intramuscular application of certain substances also affect the orthodontic tooth movement. Poosti *et al* 2009<sup>(24)</sup> studied the effect of long-term progesterone administration in 24 prepubertal female albino rabbits for nine weeks after insertion of orthodontic appliance between upper two central incisors for 18 days. A significant decrease in orthodontic tooth movement was recorded.

On the other hand, the finding of this study differ from the finding of the study carried out by Cağlaroğlu and Erdem 2012<sup>(25)</sup> who injected Prostaglandin E2 (10  $\mu$ g/mL) intravenous, submucosal, and intra ligamentous to experimental rabbits with fitted springs in between the maxillary incisors, and the study by Siefi *et al* 2003<sup>(26)</sup> who also studied the effect of submucosal inaction of prostaglandin on maxillary 1st molar movement in rats. Both studies recorded significant increases in orthodontic movement.

The result of the present study demonstrate

that, the local injection of infliximab caused a significant reduction in tooth movement in the day 5, which is considered to be due to the effect of infliximab that block the inflammatory effect of TNF- $\alpha$ <sup>(18)</sup>. In accordance with results obtained by Andrade *et al* 2007,<sup>(16)</sup>.

After that the initial movement was followed by a plateau phase (lag period) between day 5 and 9 with no significant difference between experimental and control group. Recent studies demonstrated that tooth movement may be delayed by the development of hyalinized areas and protracted osteoclast recruitment, as in accordance to search of electronic databases as well as hand searching retrieved 70 publications concerning the subject of hyalinization in search strategy from Medline, PubMed, and Embase on rat, mouse, rabbits, dog, and monkey done by Von Böhl and Kuijpers-Jagtman 2009<sup>(28)</sup> who reported that the appearance of hyalinization was recorded after 5 days. A number of explanations have been postulated for the presence of a lag period. Some have suggested that the lag is caused by nonvitalization (hyalinization) of the PDL in areas of maximal stress and that no tooth movement can occur until the area of nonvitalization has been removed by cellular processes. Others believe that the lag period may represent the interval required for absorption of the thicker compact bone of the lamina dura. hence the rate of tooth movement is reduced<sup>(29)</sup>.

After day 9, the tooth movement was re-initiated and increased steadily until day 18 in both control and experimental groups. The movement was significantly reduced in the experimental group than in control group. This result reflected the time accumulation effect of the continuous infliximab injection at day 7th and 13th till the end of orthodontic movement.

When alveolar bone loss is induced, there is a dramatic increase in leukocyte recruitment in close proximity to bone. The presence of blockers to IL-1 and TNF, decrease the recruitment of leukocytes substantially. This suggests that bone loss and periodontal disease is initiated when the inflammatory stimulus spreads to the deep gingival connective tissue, stimulating the recruitment of leukocytes. Thus, blocking TNF- $\alpha$  activities may inhibit bone loss both directly and indirectly; the latter occurring via decreased recruitment of mononuclear cells in the area of bone<sup>(21)</sup>.

The decreasing in tooth movement in this study may be related to decrease in bone resorption, since infliximab binds to soluble and membrane-bound TNF- $\alpha$  with high affinity, impairing the binding of TNF- $\alpha$  to its receptor<sup>(30)</sup>. Another action for infliximab which may play a role in the reduction of the

inflammatory reaction through killing cells that express TNF- $\alpha$  through antibody-dependent and complement-dependent cytotoxicity<sup>(31)</sup>.

The clinical result of this study need to be confirmed histologically and ultrastructurally prior to the recommendation of using local infliximab injection as an adjunctive in orthodontic treatment to reduce tooth movement especially in anchorage tooth or to control tooth movement in medically compromised patients.

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