

# Comparison of TNF- $\alpha$ Levels between Lingual & Labial Intrusion

*Running title: TNF- $\alpha$ , Miniscrew*

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## ABSTRACT

The aims of the present study was to assess the level of (TNF- $\alpha$ ) interleukin in the gingival fluid taken from around the miniscrew and the teeth during intrusion of anterior segment with labial and lingual approaches. **Material & Method:** The sample use for the study was consist from 20 male adult subjects complaining (class I, II malocclusion) with no any systemic diseases, requiring maxillary incisor intrusion with deep bite (4-5 mm), with overjet not more than (4mm), divided into two equal groups with labial fitting brackets and the other with lingual fitting brackets, two miniscrews 1.6mm in diameter with 7mm was placed at the mucogingival junction distal to the maxillary lateral incisors between the lateral and canine, palatal miniscrew was inserted (7mm) height from the main arch wire, TNF- $\alpha$  level was examine in the gingival cervicular fluid samples taken from around the teeth and miniscrews, just before treatment, 1 hour, 1<sup>st</sup> day, 7<sup>th</sup> day. **Results:** in labial and lingual control intrusion no significant differences in TNF- $\alpha$  level but there is an increase in the level of TNF- $\alpha$  after one hour from treatment start, while TNF- $\alpha$  level in around miniscrew, treated teeth in labial & lingual intrusion an increase in its level after starting the treatment (1 hour), 1<sup>st</sup> day, but returning back to decrease after 1 week with highly significant differences between the groups at significance level  $p < 0.05$ . the level of TNF- $\alpha$  at all in all comparing lingual groups are lower than that of labial groups with no significance differences at significance level  $p < 0.05$ . **Conclusion:** the effect on the level of TNF- $\alpha$  when doing intrusion of maxillary anterior segment from labial site with aids of two miniscrews between lateral and canine, the same as when use the single miniscrew adjusted 7mm high to the main arch wire in the lingual site.

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## INTRODUCTION

Intrusion defines as (anchoring of a tooth into an alveolar bone), The force that distributes alongside the root during any orthodontic tooth movement may cheer root resorption, and more resorption with intrusive force have been notice<sup>(1)</sup>. many studies conducted that's there is no any relation between the degree of intrusion and the inflammation at the apex (resorption) in normal orthodontic traditional technique (like edgewise)<sup>(2)</sup>. This risk would be greater if compared to other tooth movements and apical root resorption depends on the intensity of orthodontic movements<sup>(3)</sup>. Lingual appliances have a special biomechanics differ from the labial one and the periodontal stresses generated by orthodontic forces are transferred to the alveolar bone, leading to resorption in compressed regions<sup>(4)</sup>. Application of the same forces in labial and Lingual system in upper central incisor with altered inclinations induces different reactions so that Torque control in Labial orthodontic system was more difficult than Lingual system in each situation<sup>(5)</sup>. Considering a smaller distance between the point of force application and the center of resistance so that of a vertical force has different clinical effects on tooth movement with labial and lingual appliances and the Application of a lingual force is more complicated, and its effect on tooth movement depends on bracket position and initial tooth inclination<sup>(6)</sup>.

In lingual orthodontic treatment, torque control of the anterior teeth during space closure was achieved either by directly applying a moment and force to a lingual bracket or by using lever-arm mechanics to obtain the desired line of action of the force with respect to the center of resistance<sup>(7)</sup>.

In lingual orthodontic the immediate center of resistance for the 6 anterior teeth was located at 7.0 mm apical to the interproximal bone level between the central incisors (measured perpendicular to the occlusal plane)<sup>(8)</sup>. During the application of intrusive force labially using the mini-screws, the axial Inclination of the upper incisors showed minimal change, which was revealed to be clinically acceptable<sup>(9)</sup>

The rates of both intrusion and root resorption were higher using the anteriorly placed mini-screws, beside that no any reports have been recognized regarding incisor intrusion supported by posterior mini-implants<sup>(10)</sup>.

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), is a critical cytokine in the inflammatory response to infection, it was produced by several cell types but mainly by the monocytes/macrophages; it affects lipid metabolism, relates to insulin resistance, blood coagulation, and endothelial activity<sup>(11)</sup>. Low levels of (TNF- $\alpha$ ) seem to be related to the protection of homeostasis and to the remodeling/healing of damaged tissue through the activation and formation of fibroblasts<sup>(12)</sup>. The increase in (TNF- $\alpha$ ) level in peri-implant crevicular fluid is described to cause Peri-implantitis<sup>(13)</sup>. The aims of the present study is to assess the level of (TNF- $\alpha$ ) interleukin in the gingival fluid taken from around the miniscrew and the teeth during intrusion of anterior segment with labial and lingual approaches.

## MATERIALS & METHOD

The sample use for the study was consist from 20 male adult subjects complaining (class I, II malocclusion) with no any systemic diseases, good oral hygiene, no any history of previous root canal of anterior teeth, free from trauma for the anterior teeth requiring maxillary incisor intrusion with deep bite (4-5 mm), with overjet not more than (4mm) as the mandibular incisors would occlude lingual to the bite plane, moderate crowding (not exceed 5mm) with the incisal edge below the functioning occlusal plane, Classified to two equal groups one with labial fitting brackets and the other with lingual fitting brackets. Before insertion of mini screws, brackets (Roth technique with 0.022 slots) fixed in the labial group according to instruction, then leveling start till complete then change the full arch to sectional archwire from lateral to lateral by using (0.018\*0.022 inch) on the labial, (0.018\*0.025), on the lingual side). Self-drilling Miniscrew (Absoanchor; Dentos, Daegu, South Korea), 1.6mm in diameter with 7mm was placed at the mucogingival junction distal to the maxillary lateral incisors between the lateral and canine to reduce the root contact at right angle to the long axis of the teeth, while in the lingual region before insertion of miniscrew lateral cephalogram must take for each patient in the group to determine the center of resistance of the roots to detect exact location of mini-screw in the palate (7mm) height from the main arch wire in order to the strain on the miniscrew and reduce possibility of tipping during intrusion. The miniscrew used in the palatal region with diameter of (1.6mm) and (11mm) length was fixed in the area also approximately facing the space between the lateral and canine. Two weeks after placement, intrusive force was begun with a (150g) force delivered by power chain elastic (medium, 3M, USA) between the miniscrew and the arch wire.

The analysis of (TNF- $\alpha$ ) (MyBiosource –MB9311957 ELISA Kit) need to classified the studying samples into Labial intrusion group, lingual intrusion group from main two groups subdivided into control subgroup (represent by the premolar), miniscrew subgroup, treated subgroup for both labial and lingual groups (represented by central and lateral) respectively. The collection of gingival cervical sample was accomplished by using sterile paper point which inserted in the gingival cervical space of the teeth and in the space around the miniscrews. After isolation of the sample area with gauze and air syringe to reduce possibility of contamination, also any supra gingival plaque was carefully removed prior to sampling, were inserted into the gingival crevice until slight resistance was sensed and held in place for 60 sec, the sample taking was accomplished with (20°C) room temperature. The taking sample was done by insertion two sterile paper point in the gingival cervical space till the bottom of the sulcus for about three minutes (Fig.1), (2 $\mu$ L) is the amount of fluid that need to make the analysis, any sample contaminated with blood was discarded, then the fluid transfer to eppendorf tube all filter papers were autoclaved and weighed on a digital scale before used. Sterilized saline solution (250 $\mu$ L) was added to the Eppendorf tubes and centrifuged for 1 minute, all cytokines were recovered from the paper strips by 5 minutes of centrifugal elution, The papers were then removed and the solutions were stored at (-20°C) until the immunoassay was performed.

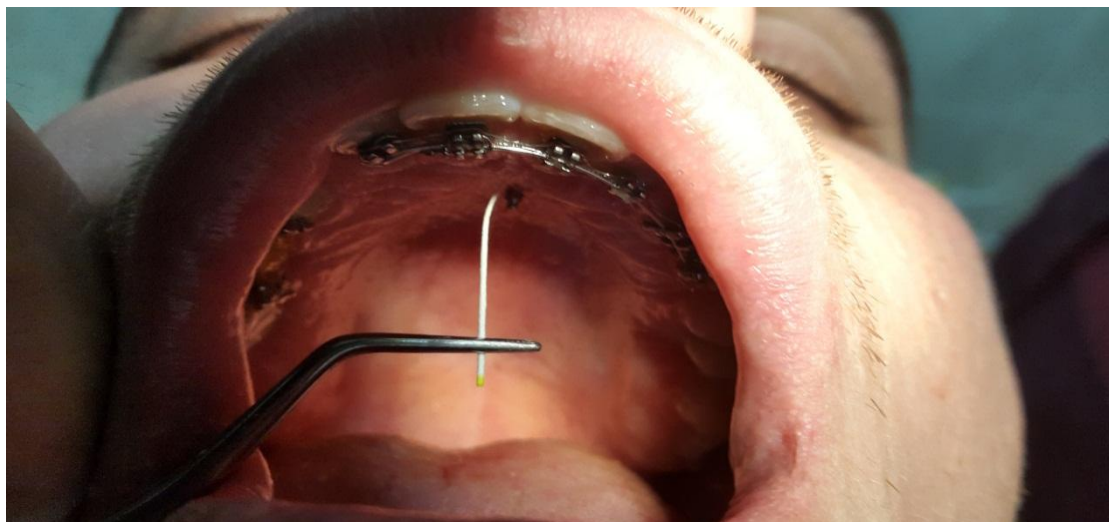


Figure (1) Sample taking from around miniscrew using sterile Paperpoint

## RESULTS & DISCUSSION

Mini-screw for intrusion mechanics has been proposed as an alternative technique to conventional mechanics<sup>(14)</sup>. The miniscrews are located in the anterior region between the central incisors, the central and lateral incisors, or the laterals and canines which is very effective as an assisted intrusion mechanics<sup>(9)</sup>. The mini-screw, in conjunction with the lever-arm, is useful not only for absolute anchorage but also for anterior torque control during retraction in lingual orthodontic treatment<sup>(15)</sup>. Chemical analysis of GCF is useful for investigating changes at a single site during a specific period and the response of dental and par dental tissues to orthodontic tooth movement. Because this is a noninvasive Method and repetitive sampling from the same side is possible, it is used especially for human studies<sup>(16)</sup>. Many studies stated that pro-inflammatory cytokines, especially TNF- $\alpha$ , show an important role in the formation and distribution of inflammation in periodontal and peri-implant structures<sup>(17)</sup>. Intrusion requires careful control of force magnitude. Light force is required because the force is concentrated in a small area at the tooth apex; Intrusion may also cause changes in the pulp tissue such as vascularization of the odontoblast and pulpal edema<sup>(18)</sup>. No significant differences in comparison of the level of TNF -  $\alpha$  in the control group of labial intrusion at time intervals, but there is an increase in the level of TNF- $\alpha$  after one hour from treatment start for both treatment and miniscrew subgroups with a significance difference when making comparison between the time interval in the treatment subgroups and highly significant differences in time interval in miniscrew subgroups at significance level  $P < 0.05$ . Besides that the level of TNF- $\alpha$  for both the treated and miniscrew subgroups shows an increase of its level after one hour and return to decrease when checked at 1 day and 1 week. Table (1)

**Table (1) Levels of TNF - $\alpha$  in the labial orthodontic**

Control labial	NO. OF Samples	Mean $\pm$ SD	Duncan's group	F - Values
Before	10	53.5 $\pm$ 10.5	A	0.955
After 1 hour	10	50.89 $\pm$ 12.91	A	
After one day	10	53.77 $\pm$ 12.48	A	
1 week	10	51.70 $\pm$ 17.09	A	
<b>Treatment labial</b>				
Before	10	103.0 $\pm$ 48.64	A	0.002*
After one hour	10	313.454 $\pm$ 149.95	B	
After one day	10	235.2 $\pm$ 113.3	B	
1 week	10	269.75 $\pm$ 136.4	B	
<b>Miniscrew</b>				
Before	10	59.22 $\pm$ 8.4	A	0.00**
After one hour	10	322.18 $\pm$ 156.9	C	
After one day	10	236.8 $\pm$ 113.42	BC	
1 week	10	171.8 $\pm$ 80.3	B	

\* Significance differs (\*\*\*) Highly Significance differs, TNF- $\alpha$  Level in (pg/ $\mu$ L)

When we notify the changes in TNF- $\alpha$  level in intrusion groups with lingual orthodontics we detect no significance differences in the level at different intervals period in control subgroup, significance differences in the levels of TNF- $\alpha$  at different period interval in the treated sub group with highly significant changes between the period intervals at the miniscrew subgroups at significance differences  $p < 0.05$  Table. (2)

**Table (2) Levels of TNF - $\alpha$  in the lingual orthodontic**

Control Lingual	NO. OF Samples	Mean $\pm$ SD	Duncan's group	F - Values
Before	10	52.8 $\pm$ 9.3	A	0.988
After 1 hour	10	51.19 $\pm$ 13.01	A	
After one day	10	52.07 $\pm$ 10.82	A	
1 week	10	51.0 $\pm$ 16.1	A	

Treatment lingual				
Before	10	86.4± 37.6	A	0.006*
After one hour	10	245.05± 129.06	B	
After one day	10	179.2± 86.6	B	
1 week	10	203.15± 105.9	B	
<b>Miniscrew</b>				
Before	10	59.52 ± 8.15	A	0.00**
After one hour	10	283.48±161.48	C	
After one day	10	211.82±110.4	BC	
1 week	10	147.5±71.86	AB	

\*Significance differs (\*\*) Highly Significance differs, TNF- $\alpha$  Level in (pg/ $\mu$ L)

The concentration of TNF- $\alpha$  increased at first 24 hours and declined at 1 week the reason for that due to the adaptation of the tissues to the light continuous force at 1 week. Our results agree with results obtained by Karacay s. et al. (2007)<sup>(19)</sup>. The increase of TNF- $\alpha$  Level at 1 hour was probably because of the incomplete diffusion of the cytokine into the sulcus in 1 hour, resulting in an acceleration of the production of inflammatory cytokines. This interpretation agrees with the results obtained by Ozaki S, et al. (2005)<sup>(20)</sup>. When make comparison in labial and lingual orthodontic intrusion at time period interval between control, treated and miniscrew on both sides, we record a significance differences between the groups at different time interval with higher mean values in level of TNF- $\alpha$  in all labial groups when compare to lingual groups, the rapid and more production of TNF- $\alpha$  levels in labial groups explain as the intrusion we need in labial technique exerts more force than in lingual technique so more alkaline phosphatase (ALP) activity, resulting in an acceleration of the production of inflammatory cytokines, thus increase in the production of TNF- $\alpha$  Levels more than in lingual orthodontics. These results agree with results obtained by Ozaki S, et al. (2005)<sup>(20)</sup>, Karacay s. et al. (2007)<sup>(19)</sup>. The results of the present study regarding the increase in the level of TNF- $\alpha$  at first hour and 7 days agree with result obtained by Başaran et al. (2006)<sup>(21)</sup>. The results of the present study shows that the levels of TNF- $\alpha$  around the miniscrew was higher than that around the teeth for both the lingual and labial intrusion techniques and these results agree with that obtained by Nowzari et al. (2008)<sup>(22)</sup>. The results of the present study shows that there is increase in the level of TNF- $\alpha$  around both the teeth subjected to orthodontic intrusion and the miniscrews in the initial periods at first (1 hour, 1st day) then start to decline (when observe at 7<sup>th</sup> day), these changes can explained as the intrusive force applied, there is an acute response to this force, then the declination in the level can be discuss it as adaptation of the periodontal tissue to orthodontic intrusive forces, and the next reaction or response to additional force pointed by less harmful effect due to this adaptation of periodontal reaction. These Results agree with results obtained by Jäger et al. (2005)<sup>(23)</sup>.

In the present study the differences in the level of TNF- $\alpha$  around the teeth and mini-screws at different period interval for both lingual and labial intrusion techniques we notify that the mean level of cytokine in the lingual technique was less than that of the labial technique but no significance differences among all groups Table(3)

In labial orthodontic intrusive force the net vector force pass through the center of resistance while in lingual orthodontic intrusive force pass beyond the center of resistance so the amount of intrusive force that need is less than that in labial orthodontic beside that a vertical force applied with labial and lingual appliances has different clinical effects on tooth movement. Thus in lingual force, it is more complicated, and its effect on tooth movement cannot be exactly expected because it depends on bracket position and initial tooth inclination<sup>(6)</sup>. So that these points might explain why the level of cytokine approximate the same for both lingual and labial intrusion around the treated teeth and miniscrews in addition to the criteria of the cases selected for the research that may need less force required for intrusion. In additions the use of miniscrew in the labial position between the lateral and canine approximate the center of resistance produce more intrusive force less flaring of anterior teeth and more resorption when compared with posteriorly placed miniscrew<sup>(24)</sup>. Beside that a study conducted by omur et al. (2009)<sup>(9)</sup> agree with the present study result in that Rootresorption was not seen as a consequence of Incisor intrusion and there is no great force applied during intrusion.

**Table 3: Comparison of TNF- $\alpha$  Level between labial and lingual orthodontic**

Control	Mean ± SD Labial orthodontic	Mean ± SD Lingual orthodontic	F – Values
Before	53.5±10.5	52.8± 9.3	0.877



After 1 hour	50.89±12.91	51.19±13.01	0.959
After one day	53.77±12.48	52.07±10.82	0.749
1 week	51.70±17.09	51.0± 16.1	0.926
<b>Treatment</b>			
Before	103.0±48.64	86.4± 37.6	0.04*
After one hour	313.454±149.95	245.05± 129.06	0.289
After one day	235.2 ±113.3	179.2± 86.6	0.231
1 week	269.75± 136.4	203.15± 105.9	0.239
<b>Miniscrew</b>			
Before	59.22± 8.4	59.52 ± 8.15	0.936
After one hour	322.18±156.9	283.48±161.48	0.594
After one day	236.8±113.42	211.82±110.4	0.624
1 week	171.8± 80.3	147.5±71.86	<b>0.485</b>

\*Significance differs ,TNF- $\alpha$  Level in (pg/ $\mu$ L)

There is an agreement with the present study in the stresses were significantly less for the lingualside force application compared to the labial side for intrusion as presented by <sup>(25)</sup>. The results of the present study agree with results obtain by GeramyA & Sheikhzadeh S. (2015), they state that the Torque control in Labial orthodonticsystem was more difficult than Lingual system in eachSituation<sup>(26)</sup>. Beside that the insertion of miniscrew in the palate 7mm above the main arch wire in combination with lever-arm can also be used to controlthe point of force application in the posterior area and producethe ideal force system in lingual orthodontic<sup>(15)</sup>.

## CONCLUSION

The effect on the level of TNF- $\alpha$  when doing intrusion of maxillary anterior segment from labial site with aids of two miniscrews between lateral and canine , the same as when use the single miniscrew adjusted 7mm high to the main arch wire in the lingual site.

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