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ABSTRACT
Background: The role of periodontal mechanoreceptors (PMRs) in the reflex control of the jaw muscles has been mainly derived from animal studies and few human studies. This study investigated the response of the PMRs in the activity of the masseter muscles in humans to controlled orthogonal stimulation of the upper left central incisor, both before and during orthodontic treatment.

Materials and methods: Surface EMG of sixteen neurologically normal male subjects (mean age 13.2) were recorded following a rapid stimuli of 3 Newtons. 8 class II patients with retrognathic mandible were treated with twin block and 8 class I patients were treated with pre adjusted fixed appliance. Pre-treatment and during treatment EMG of masseter were recorded in both the groups in three stages namely without stimulus in rest position, with stimulus in rest position without splint and with both stimulus and splint.

Results: The class I subjects showed a statistically significant (p<0.05) decrease in latency period when compared to class II subjects. This showed that the class I malocclusions had quicker excitation of PMR stimulated masseteric reflex when compared to class II div I malocclusion. Both the groups showed variations in post-treatment post-stimulus response when compared to pre-treatment response. There was a statistically significant reduced latency period.

Conclusions: This study has shown that periodontal mechanoreceptors supply information regarding position and forces applied to the tooth. Large scale studies are needed to validate these findings.

Key Words: Periodontal mechanoreceptors, Masseteric reflex and tooth movement.

INTRODUCTION
The connective tissue structure, periodontal ligament [PDL] suspends teeth in their socket by linking the cementum and alveolar bone. With diverse functions, PDL is innervated richly by nerve endings and receptors that are involved in proprioception and neuromuscular reflex. The application of orthodontic force induces a cascade of biophysical, bioelectrical and biochemical changes which results in alveolar translocation, where in the tooth along with its attachment apparatus is moved from one position to another. The orthodontic appliance therapy remodels PDL and alveolar bone simultaneous to the altered teeth position. Numerous studies had extensively dealt with these tissue changes observed in PDL, bone tissues and TMJ. 1-4 McNamara stated that during functional jaw orthopaedic treatment the orofacial system undergoes considerable change and the mandible presumably attains an altered functional position. 5 Occlusal alterations also result from modifications of respective skeletal bases and from migration and tipping of teeth in the maxillary and mandibular dentitions. Subsequent to the remodeling of PDL during orthodontic tooth movement, it is logical to assume that function and or response of mechanoreceptors could be interfered during or after orthodontic treatment. The neuromuscular reflex pattern changes following the teeth movement is a poorly elucidated topic. There are little studies regarding the neuromuscular reflex and its relation to positional changes in tooth. The available literatures are mostly limited to animal studies. 6-8 The scientific literature is silent in this aspect of research and therefore this study was done to highlight the role of periodontal mechanoreceptors in reflex control of human masticatory system and also following orthodontic treatment. Stimulation of the periodontal mechanoreceptors can be achieved by mechanical means and the easiest way to mechanically stimulate teeth is by means of tapping the labial surface of incisor teeth with a probe (orthogonal stimulation).

Experiments in animals indicate that a large amount of feedback for jaw-closing muscles comes from periodontal mechanoreceptors (PMRs). 9, 10 Human reflex studies can be used to investigate the synaptic connections between afferent systems and moto neurons. The principle muscle reflexes evoked by stimulation of periodontal mechanoreceptors involve only the jaw closing muscles and the measurement can be done by
The study was to study the role of PDL mechanoreceptors in neuromuscular reflex after orthogonal stimulation on the labial surface of the central incisor before and during orthodontic and functional jaw orthopaedic treatment. The contribution of PMR is assessed by the reflex response of human masseter reflex control by stimulated periodontal mechanoreceptors using surface electromyogram (EMG).

**METHODS**

Ethical approval. Written informed consent was obtained from all the subjects who participated in the study. The protocol was approved by the Institutional Ethics committee and all procedures used conformed to the Declaration of Helsinki.

Subjects. Sixteen neurologically normal male subjects with healthy teeth and gums, and no history of orthodontic treatment or dysfunction aged between 9–15 years (mean age of 13.2) participated in the study. Consumption of analgesics 24 hours before the study was not allowed. A total of 37 participant records were initially reviewed. Eleven participants were excluded because they failed to meet the inclusion criteria and therefore did not participate in the experimental session. Six participated in the recording session, but due to reasons like non-compliance in wearing of the functional appliance were excluded. Four participants were excluded due to insufficient signals, not following directions, or because three stage sequences could not be visually identified in the signal. Therefore, a total of sixteen participants were available for inclusion in the current study.

The sample was divided into 2 groups of 8 subjects each. Group 1 had Angles class II div I malocclusion on a class II skeletal base with retrognathic mandible and Group 2 had Angles class I malocclusion with increased over jet on a class I skeletal base. All these subjects had reported for correction of malocclusion. The study models were prepared and the routine clinical photographs and lateral cephalometric radiographs were taken for the subjects both before and during the orthodontic appliance therapy.

General protocol. The bilateral surface EMG recording of masseter was done after mechanical stimulation prior to and during orthodontic treatment. The group 1 subjects underwent Twin block Functional appliance therapy with 6 mm advanced construction bite and vertical opening of 2mm above the freeway space. The group 2 subjects underwent extraction of bilateral maxillary first premolar and treatment with pre-adjusted edgewise fixed appliance therapy as part of routine orthodontic treatment. The subjects were made to be seated in postural rest position with Frankfort horizontal plane parallel to the floor. A fixed nosepiece was used to minimize the movement of the subject’s head, which also counteracted the axial forces applied to the tooth. The masseter muscle EMG location was determined by palpating the area while the participant clenched and relaxed jaw muscles. The skin around the area of the masseter muscle was then cleaned with an alcohol wipe to clear away any debris or dead skin. Then, a piece of biomedical tape was dabbed on the area to remove any excess debris or dead skin. Finally, an adhesive interface was placed on the sensor and the sensor was

<table>
<thead>
<tr>
<th></th>
<th>Rt. masseter Without splint</th>
<th>Lft. masseter Without splint</th>
<th>Lft. masseter With splint</th>
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<tr>
<td><strong>Pre-treatment</strong></td>
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<tr>
<td>Group 1</td>
<td>350ms, 21ms</td>
<td>350ms, 16ms</td>
<td>200ms, 20ms</td>
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<td></td>
<td>250ms, 31ms</td>
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<td>Group 2</td>
<td>200ms, 17ms</td>
<td>275ms, 17ms</td>
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<td><strong>Post-treatment</strong></td>
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<tr>
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<td>200ms, 20ms</td>
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<td>250ms, 12ms</td>
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<tr>
<td>Group 2</td>
<td>250ms, 18ms</td>
<td>200ms, 13ms</td>
<td>200ms, 9ms</td>
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<td>200ms, 12ms</td>
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The silent period was followed by an inhibitory response with a latency period.
Periodontal Mechanoreceptors Stimulated Study of Human Masseter Reflex Control

then placed onto the various areas. Silver chloride gel was applied on the bilateral masseter muscles. Surface electrodes were placed on the skin overlying the left and right masseter muscles in order to detect the SEMG activity. Grounding of the subject was achieved by the use of a hand wrist electrode and mastoid bone was used as the bony area of reference (Figure 1). The masseter reflex activity was recorded using 9mm wide electrode in 3 stages . The EMG of normal masseter reflex in this rest position was recorded (stage 1). Without changing the postural rest position, the orthogonal mechanical stimulation was given to the maxillary left central incisor on its labial surface by an impact hammer of digital storage oscilloscope which ensured uniform force of 3 Newton and the EMG activity was recorded (stage 2). The stimulus was delivered after the start of the oscilloscope sweep. For uniform posterior disclusion, a 3mm thick acrylic occlusal splint with relief on labial aspect and covering on lingual aspect was used. The EMG activity was recorded after mechanical stimulation on the labial surface of the same incisor with occlusal splint and in the same postural rest position (stage 3). To avoid change in EMG due to anticipation of the stimulus, it was necessary to apply the stimuli at random intervals so the subject could not predict when they would occur. All the EMG recordings were amplified, full wave rectified, filtered with sensitivity of 20mV, Frequency 50Hz-500Hz and stored in the computer. The entire procedure was repeated after 6 weeks of orthodontic treatment. The changes in the incisor position were also recorded after 6 weeks in both groups. The results were tabulated (Table 1) and statistically analyzed.

Statistics. T-test was done to calculate the p-value for the records within group 1 right and left latency periods both with and without splints were tabulated (Table 1) and statistically analyzed. Group 1 and 2 subjects. If the p value calculated is < .05 it was considered statistically significant. Correlation test been done with Pearson 2-tailed test to correlate significance difference within group1 and group2. The significance was set at the level of .01 and .05 respectively. Paired T-Test was done to compare pre-treatment and post treatment changes of group 1 and group 2. NPar Tests- Wilcoxon Signed Ranks Test were used which was a non-parametric test that compared the median of a column of numbers against a hypothetical median. In our study it was done for the post and pre-treatment silent period values which exceeded normal mean parameters. The data collected were assessed using SPSS statistical software (version 20.0, SPSS Inc., Chicago, IL, USA).

RESULTS

The Pearson 2-tailed test was used to correlate significance difference within groups 1 and 2 and the significance were at the level of .01 and .05 respectively. There was statistical significance in pre-treatment records within group 1 (at 0.01 level) and within group 2 (at 0.05 level) right and left latency periods with and without splint. There was statistical significance in post-treatment records within group 1 at 0.05 level (table 2) and within group 2 at 0.05 level (table 3) right and left latency periods without and with splint (Figure 2 and 3). However in group 2, the latency period was decreased in left side both with splint and without splint. The p-value of groups 1 and 2 subjects were done using T-test and values less than 0.05 were considered statistically significant. There was a statistical significance between group 1 and group 2 in the pre-treatment latency period at p <0.05. There was a decrease in the latency period in group 2 [Rt-21.13, Lt-

<table>
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<tr>
<th>Correlations</th>
<th>Rt - Post Treatment</th>
<th>Lt - Post Treatment</th>
<th>RS - Post Treatment</th>
<th>LS - Post Treatment</th>
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<td>Sig. (2-tailed)</td>
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<tr>
<td>RS - Post Treatment</td>
<td>Pearson Correlation</td>
<td>.599</td>
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<td>Sig. (2-tailed)</td>
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<tr>
<td>LS - Post Treatment</td>
<td>Pearson Correlation</td>
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<td>Sig. (2-tailed)</td>
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*Correlation is significant at the 0.05 level (2-tailed). There was a statistical significant difference in post treatment records within group 1 right and left latency periods both with and without splints.
There was no statistical significance between group 1 and 2 in post treatment at \( p > 0.05 \), with and without splint. The paired T-Test was done to compare pre-treatment and post-treatment changes of the subjects of both groups. The pattern of masseteric reflex in the post-treatment of group 1 and group 2 was same as pre-treatment of group 1 and group 2 at \( p > 0.05 \). There was no statistical difference between pre-treatment silent period in group 1 and also in group 2 subjects at \( p > 0.05 \); and between post-treatment silent period in group 1 and group 2 (\( p > 0.05 \)).

The NPar Test- Wilcoxon Signed Ranks Test were used in this study to compare the silent period values that exceeded normal mean parameters between the before and after treatment initiation stages of the subjects. There was a statistical significance in the post-treatment silent period within group 2 right and left latency periods with and without splint. The latency period is decreased in left side both with splint and without splint.

**DISCUSSION**

Periodontal mechanoreceptors are special kind of receptors which are directional sensitive and they respond more to the direction of force and also temperature sensitive. Human periodontal mechanoreceptive afferents innervating the anterior teeth respond to loads applied to the teeth and are all slowly adapting. They are active spontaneously and discharge regularly in response to forces applied to teeth. They are similar to type II mechanoreceptors of skin, which are slow adapting in nature. The periodontal mechanoreceptors can be stimulated when the tooth is moved within the alveolar bone. Mechanical stimulatory force delivered more than 5N and it stimulated the stretch receptors of the muscles and influenced the reflex response. Therefore in this present study the mechanical stimulation given was brisk taps which were able to deliver force of 3N which had been standardized by means of digital storage oscilloscope. The effects of most of the stimuli used in the present study have been localized to receptors in the periodontal area.

The general pattern of response of masseter to the mechanical stimulation is that of a silent period which was followed by an inhibitory response. The averaged electromyography response in this study showed a characteristic inhibitory response following a silent period with a latency of about 18ms. The time gap between the inhibitory and excitatory response constitute the latency period. The latency period will be followed by an excitatory reflex which occurs due to actual contraction of masseter muscle and causes closure of mouth. In this study, the excitatory reflex could not be elicited because the subjects were prevented by the splints from closing the mouth. Also the findings were recorded only in postural rest position. This kind of neuromuscular reflex following stimulation of periodontal mechanoreceptors without the excitatory reflex considered as periodontal masseter reflex to distinguish from jaw closing or jaw jerk reflex. The inhibitory response seen following the mechanical stimulation is essentially a protective reflex. This serves to reduce the activity of the jaw closing muscles when a person bites unexpectedly on heavy objects.
Fig.1 Diagrammatic representation of the experimental set up. The subjects bit into the acrylic splint, with relief on the labial surface. Movement of the subject was further minimized by the use of a fixed nosepiece. A digital storage oscilloscope then produced the desired force of 3 N. The bony reference area selected was the mastoid bone (the electrodes kept behind the ears). The bilateral masseter EMG was amplified, full wave rectified, filtered (band width 20-50Hz) with sensitivity of 20mV, High frequency- 50Hz and low frequency 500Hz, and stored in the computer.

Analysis of pre-treatment reflex findings showed that experimental side (left masseter) showed statistically significant increased silent period followed by a reduction in latency period. These findings coincide with that of O’Connor SJ and Türker KS. The mechanical stimulation of human tooth evokes and increased silent period and there is a transient excitation of periodontal masseteric reflex which results in reduction of latency period. Similar results were obtained in both group 1 and group 2 subjects following mechanical stimulation. Another significant finding was the difference in masseteric reflex response between subjects with class II div I malocclusion (group 1) and class I malocclusion (group 2). Group 2 subjects showed a statistically significant (p<0.05) decrease in latency period when compared to group 1 subjects. The reduction in latency period among subjects with class I malocclusions showed that these subjects had quicker excitation of masseteric reflex when compared to slower excitation among subjects with class II div I malocclusion. The pattern of masseter EMG reflex in the post treatment was the same as pre-treatment silent period followed by inhibitory reflex has shown as latency period.

But both group 1 and group 2 subjects showed variations in post treatment post stimulus response when compared to pre-treatment response. Silent period showed no significant difference, but there was a short latency inhibitory reflex response shown by a statistically significant reduction in latency period. This showed that application of orthodontic force did not change the occurrence of masseteric reflex.

There was a definite change in the response of the masseter muscle to the periodontal mechanoreceptors. The reflex inhibition period was shorter in post treatment reflex. This result is in contrast to the finding of a study by authors. This could be attributed to the difference in method of stimulation. In this study orthogonal stimulation was employed in rest position in contrast to axial stimulation followed by closure of mouth in their study by the authors. No significant changes were observed when post treatment post stimulus response of group 1 and group 2 were compared.

Though there was unilateral mechanical stimulus applied on the left side, the post stimulus response of the right side showed variation from pre stimulus recording in this study. This finding correlates with the findings of a previous study, who found that unilateral electrical stimulus, stimulates bilateral masseteric moto neurons.

This study has shown that periodontal mechanoreceptors supply information regarding position and forces applied to the tooth in accordance with a previous study by authors who discovered that the strength of the inhibitory reflex response to a tooth-tap stimulus was much larger than previously stated and that periodontal mechanoreceptors are extremely sensitive tactile sensors present in natural teeth. These sensors provide information about tooth loads and are located among the collagen fibres in the periodontal ligament. The changes observed in the study indicated that orthodontic force can induce functional changes in the periodontal mechanoreceptors, which was evident in the altered response following application of
orthodontic force. It remains to be seen whether the altered response is a transient phenomenon and will it revert to original state after completion of orthodontic treatment. Another interesting finding to be explored further is the difference observed in the periodontal masseteric reflex response seen in class I and Class II div I of malocclusion.

This study was carried out by applying rapid orthogonal stimulation of tooth. Axial stimulation of tooth, slow force stimulus, other receptors like muscle spindles, acoustic receptors and TMJ receptors can also contribute to the response of periodontal mechanoreceptors. Further studies on the factors can throw new information on the role of PMRs in neuromuscular reflex pathways.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST STATEMENT

None to declare.

REFERENCES