

Evaluation of Gingival Inflammation, Plasma IL-6, and TMJ Clicking after Masseters Muscle Botulinum Toxin Injection and Intra-Articular PRP Injection In Bruxism Patients

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Abstract

In this study, thirty patients complaining of bruxism at night and hyperactivity masseters muscle causing TMJ disk changes, TMJ clicking sound and all selected patient complaining of gingival inflammation as included criteria, and 50-unit botulinum toxin type A were injected in both side of masseter muscle. Regarding to T.M.J clicking sound there were three intervals of PRP injection for 3 months. The levels of IL-6 in the blood samples measured by ELISA technique, this study proposed to investigate the plasma IL-6 level in patient with early changes of T.M.J disorder and role of repetitive intraarticular PRP injection on the T.M.J clicking sound and the effect of decreasing stress force microtruma of masseter muscle on gingival score in previously diagnosed bruxism patient with gingival inflammation whether of causative factor of gingivitis. The mean difference of plasma IL-6 in between patient and control (25.6 ± 4.2 and 23.8 ± 1.8) respectively. There were highly significant differences in gingival index score of all sided of oral cavity after botox intramuscular injection. Force stress muscle microtruma of bruxism patient considered a contributory factor that increased gingival inflammation these fact suggested by botulinum toxin injection to decrease this force, and highly statically difference on gingival score after injection and also, the intraarticular injection of PRP in T.M.J disorder patient statically not affected on the clicking sound of joint as well as suggesting in our research for further studies to appear the role of IL-6 in early joint changes locally as an experimental study.

Keywords : *Gingival Inflammation, IL-6, TMJ, botulinum toxin, PRP , Bruxism*

Introduction

Bruxism is defined as non-desirable upper and lower teeth contact that causing in clenching or grinding of teeth due to repetitive, unconscious tightening of the masseter and temporalis muscles⁽¹⁾. One of the biggest problem faced the dentist in the daily work is the fracture of dental restoration, many dentists forget the force from excessive teeth contact as in bruxism that causes teeth wearing, fracture, tooth cracking and gingival,

periodontal damage and tooth mobility⁽²⁾.

The etiology of temporomandibular joint disorder stilled unknown, but many factors included, one of these factors occlusal interference that contributed in masseter muscle over contraction and spasm and these microtrauma that causing temporomandibular disorder and pain was considered the most obvious symptoms.

Proinflammatory cytokines play an important role in inflammation and dysfunction of the joint and associated structure as well as muscle of mastication especially lateral pterygoid muscles and masseter. Interleukin-6 (IL-6), IL-1 and TNF-a, have been important roles implicated in pain and tenderness by developing and maintenance of muscular hyperalgesia and also

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increased level of proinflammatory cytokines leading to express psychological stresses and depression in patient with TMJ disorder⁽³⁾.

Parafunctional habits have been thought to cause TMJ microtrauma or masticatory muscle hyperactivity; however, these habits are also common in asymptomatic patients. Although parafunctional habits may play a role in initiating or perpetuating symptoms in some patients, the cause-and-effect relationship remains uncertain. There is some evidence to suggest that anxiety, stress, and other emotional disturbances may exacerbate TMJ disorders, especially in patients who experience chronic pain. It is not clear which symptoms are more common in which TMJ disorders; however, it is generally assumed that joint clicking or grating signifies intra-articular derangement whereas headache, neck pain⁽⁴⁾. Clicking sounds (a single sound) have energy concentrated in a short time interval (10–100 ms), while crepitus (a series of sounds) is longer in duration (0.1–1 s). Various attempts to characterize TMJ sounds have been made. Watt described waveforms of clicks and crepitus based on the oscilloscopic display of the analog sound recordings. Reduction of the articular disc has been suggested as one of the major causes of TMJ clicks⁽⁵⁾.

Methods

In this study, thirty patients complaining of bruxism at night and hyperactivity masseters muscle causing TMJ disk changes, TMJ clicking sound and all selected patient complaining of gingival inflammation as including criteria, all of those patient diagnosed in private clinic of oral medicine specialist in Najaf City private dental center in period from 25/4/2020 to 15/2/2021.

All of patients informed the purpose of study and taking her consent, intraoral examination has been done and all of the gingival index score were obtained by specialist of dental prevention in this center, after extra

and intraoral examination and including criteria that all selected patient without any sign and systemic disease were approved 50-unit botulinum toxin type A diluted in 2 ml distilled water and injections of 5 unit of diluted botox in each one of 3 marked point one the masseter muscle in both side below the line from angle of mouth to tragus of ear, botulinum toxin prevented release of acetylcholine neurotransmitter in neuromuscular junction and resulting in muscle paralysis and decrease of muscle tone, after 15 days of botulinum toxin injection gingival index score were also measured.

Temporomandibular joint clicking sound were hearing by stethoscope for each patient, all of patient injected by platelet rich plasma (PRP) made from 5 ml Venus blood in PRP tube as described by Mazzocca et al.⁽⁶⁾.

The buffy coat was resulted from centrifugation at the superior surface of fluid in PRP tube and red blood cell at the bottom, the procedure was finished and 2/3 of poor plasma from platelet removed by syringe and late about 15 minute to insure the buffy coat was linked and dissolved after gentle shaking the tube in the area of visible gel. Finally, 1ml of PRP aspirated by syringe. Aspirated PRP injected in the TMJ joint in point below 2mm under the 10 mm line drawing from tragus to canthus of the eye. This procedure of PRP injection was done after aspiration to avoid auriculotemporal artery injection^(7,8).

Three interval of injection for 3 month and joint sound were evaluated at the end of two weeks from last month. ELISA assays were performed to determine expression levels IL-6 in the blood samples, according to manufacturer instructions (BT LTD company Research Institute). Limitation of present study difficulty in patient follow-up the research begun with thirty patients and dropped to 25 patients in 3 months.

Result

Table (1): Descriptive statistics and differences in age and BMI between patients and control

	Patients	Control	df	T - test	P value
Age (Years)	26.78 ± 6.99	25.78 ± 6.69	44	0.49	0.62
BMI (Kg/m ²)	30.29 ± 2.85	29.74 ± 3.01	44	0.60	0.54

In table (1) the mean age and body mass index between patients and controls were non-significant difference identified by applying independent t-test.

Table (2): Differences in IL-6 level of Pre-injection patients, Post-injection patients and control groups

IL-6 Level	Mean ± SD	F-test	P value
Pre	25.6 ± 4.2 A	12.3	0.07
Post	20.3 ± 1.2 B		
Control	23.8 ± 1.8 C		

Different letters refer to high significant difference at P value < 0.01; SD: Standard Deviation

In table (2), the data of plasma level of IL-6 was calculated by applying F-test that comparing in between groups. There are non-significant differences p value 0.07.

Table (3): Differences in joint sound of patients before and after intra-articular PRP injection

Joint clicking		Post		Chi-test	P value
		Positive	Negative	12.3	0.06
Pre	Positive	12	11		
	Negative	0	0		

In table (3), the data joint sound was measured by using Chi-test that. There are non-significant differences p value 0.06.

Table (4): Differences in gingival index score in the molar region of the left lower jaw of patients before and botox injection

	Pre Patients (MS ±SD)	Post Patients (MS ±SD)	n	Z test	P value
gingival index score	1.47 ± 0.51	0.34 ± 0.48	19	3.82	0.000 *

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (4), gingival index score in the molar left region of lower jaw was statistically highly difference after botox injection with p value 0.000

Table (5): Differences in gingival index score in the molar region of the right lower jaw of patients before and botox injection

	Pre Patients (MS ±SD)	Post Patients (MS ±SD)	n	Z test	P value
gingival index score	1.26 ± 0.54	0.13 ± 0.34	21	4.01	0.000 *

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (5), gingival index score in the molar right region of lower jaw was statistically highly difference after botox injection with p value 0.000

Table (6): Differences in gingival index score in the molar region of the left upper jaw of patients before and botox injection

	Pre Patients (MS ±SD)	Post Patients (MS ±SD)	n	Z test	P value
gingival index score	1.43± 0.50	0.30 ± 0.47	21	4.01	0.000 *

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (6), gingival index score in the molar left region of upper jaw was statistically highly difference after botox injection with p value 0.000

Table (7): Differences in gingival index score in the molar region of the right upper jaw of patients before and botox injection

	Pre Patients (MS ±SD)	Post Patients (MS ±SD)	n	Z test	P value
gingival index score	1.60± 0.65	0.21 ± 0.42	20	3.92	0.000 *

MS : Mean of Scores ; SD : Standard Deviation ; * p < 0.01 : High significant difference by Wilcoxon Signed Rank Test

In table (7), gingival index score in the molar right region of upper jaw was statistically highly difference after botox injection with p value 0.000

Discussion

It has been suggested that trauma from occlusion in the presence of periodontal inflammation may be an important contributory factor in the pathogenesis of periodontal disease^(9,10). Thus, occlusal forces may alter the path of spread of gingival inflammation and thereby facilitate direct penetration into the periodontal ligament enhancing angular bone resorption and infra bony pocket formation^(11,12).

Therefore, in our study highly significant differences in gingival inflammation after botulinum toxin injection in masseter muscle suggesting decrease level of force applied on teeth by muscles botox injection lead to decrease spreading and penetration of gingival inflammation as well as the considering bruxism as causative factors or contributory factor in gingivitis and needing further studies in futures.

The importance of IL-6 in OA is also supported by previous results. It was shown that IL-6 concentrations in synovial fluid were considerably higher in patients with cartilage defect or OA than in donors without joint pathology. Follow-up study showed that increased serum concentrations of IL-6 were associated with articular changes observed in radiographs⁽¹³⁾.

In present study there is no significant in plasma IL-6 level in patient with T.M.J changes as a clicking sound and our suggestion that local changes of cytokines in early stage of T.M.J disorder and changes occur without developing pain response that result agree with previous research⁽¹⁴⁾.

Pain is one of the common sensations which usually indicates tissue damage as a consequence of various external or internal factors. From the results of previous report, it seems that masticatory muscles are capable to adapt to minor occlusal changes and chronic stress without developing the changes in pain response, but when this two proposed etiological factors work together

they cause a higher pain response⁽¹⁴⁾.

In the present study, similarly to pain response results, there was a lack of significant changes in both tissue and plasma IL-6 levels between the chronically stressed animals and rats with occlusal interference in comparison to the control group, while masseter IL-6 significantly increased in animals submitted to combination of experimental procedures. These results suggest that only the combination of occlusal interference and chronic stress leads to a strong local inflammatory reaction characterized by the alteration in masseter IL-6 level. Results of other studies showed increased levels of IL-6, prostaglandins, serotonin, and growth factors in muscle tissue after the contraction and muscle damage⁽¹⁵⁾. Also, cytokines (IL-6, IL-1, IL-8, TNF-a) and prostaglandins, when locally injected, influence nociception causing hyperalgesia⁽¹⁶⁾.

Contrary to positive association between pain response and masseter muscle IL-6 level found in this study, in most plasma samples the IL-6 level was below the detectable limit of the assay and there were no differences in plasma IL-6 levels between groups as well as no correlation with pain. Although not significant, there was a negative correlation coefficient between the pain and blood IL-6 level. Low level of blood IL-6 found in this study, especially in the group with combination of occlusal interference and chronic stress and negative correlation coefficient regarding to pain response point to the difference between local and systemic cytokine response. It has been suggested that blood IL-6 level does not depend on its local level and in contrarily to local proinflammatory reaction, the systemic cytokine response might be directed towards suppression of inflammatory reaction⁽¹⁷⁾.

After 3 intervals of PRP injection there were no significant differences statically in between group and that came in agreement with previous studies stated that the joint sound was evaluated using VAS scale⁽¹⁸⁾ other studies it was calculated on joints affected by sound or crepitus,⁽¹⁹⁾ and in another two number of patients reporting sound was scored⁽²⁰⁾. In all of the

works analysed joint sound was found to improve during follow up. But remained results were not statistically significant, nevertheless an improvement was noticed (20).

Conclusion

force stress muscle microtruma of bruxism patient considered a contributory factor that increased gingival inflammation these fact suggested by botulinum toxin injection to decrease this force, and highly statically difference on gingival score after injection and also, the intraarticular injection of PRP in T.M.J disorder patient statically not affected on the clicking sound of joint as well as suggesting in our research for further studies to appear the role of IL-6 in early joint changes locally as an experimental study.

Ethical Clearance : Taken from University of Kufa ethical committee

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Conflict of Interest : Nil

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