EFFECT OF INJECTION BOTOX IN MASSETER MUSCLE ON MORPHOMETRIC MEASUREMENTS OF THE MANDIBLE IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To study the effects of injection botox in masseter muscle on the morphometric measurement of underlying mandible and total body weight change in Sprague Dawley rats.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Anatomy Department, Army Medical College, Rawalpindi, with collaboration with Military Hospital, Rawalpindi and National Institute of Health (NIH), Islamabad, from Jun to Aug 2020.

Methodology: Female sprague dawley rats, 10 weeks of age, weighing 200-250 gm were selected. They were housed in separate cages in animal house of NIH under standard laboratory conditions and received regular diet and water ad libitum. Animals were divided into three groups. Group-A served as control while group B and C were experimental groups. Group-B was sham injected whilst group-C was injected by injection botox in left masseter muscle. Initial and final weights of the rats were recorded. All the rats were sacrificed after completion of experimental period. Weight of left mandibles was recorded. All the specimens of mandibles were cleaned and the mandibular length, condylar head length and width were measured by Vernier caliper.

Result: Total 30 sprague dawley rats were included in the study. Injection botox decreased the morphometric measurements of the mandible in experimental group-C when compared with control group-A and sham group-B with the statistically significant result (p<0.001).

Keywords: Botox, Bone loss, Mandible, Morphometric measurements.

How to Cite This Article: Iram *M, Kiani MRB, Qamar K, Saleem S, Atta MF, Zakria I. Effect of Injection Botox in Masseter Muscle on Morphometric Measurements of the Mandible in Sprague Dawley Rats. Pak Armed Forces Med J 2022; 72(1): 8-11.*

INTRODUCTION

Botulinum toxin type A is the most active type of neurotoxins produced by bacterium Clostridium botulinum. This neurotoxin inhibits the release of acetylcholine from the motor neuron, disentangling the neuromuscular junction leading to transient paralysis of the skeletal muscle, lasting for approximately 4-6 months. Paralysis, fatigue, and atrophy are the symptoms of botulinum toxin type A in skeletal muscles.[1,2]

In the musculoskeletal system, precise balance between muscles and bones is maintained, in which both the tissues assist each other by producing biomechanical force and biochemical signals. Thus the structure and function of the corresponding bone is disrupted when muscle paralysis is induced by the injection of botulinum toxin type A1. The recent use of type A botulinum toxin in aesthetic medicine to relieve wrinkles and for facial muscle reshaping is on the rise.[3] A report from the American Society of Plastic Surgeons showed a rise in body shaping and non-invasive procedures, with figures showing that minimally invasive cosmetic procedures have increased by almost 200% since 2000. Among the 15.7 million minimally invasive procedures conducted in 2017, botulinum toxin type A is the most commonly used.[4] It is used therapeutically in certain conditions such as spasmodic dysphonia, voice tremors, stammering, vocaltics, headaches, cervical dystonia, myalgia, trigeminal neuralgia, chronic neck pain, sialorrhoea, bruxism and oromandibular dystonia.[5] Cosmetically it is utilized in patients with masseter muscle hypertrophy and reducing the appearance of facial wrinkles, muscle bands formed in platysma, strabismus, blepharospasm, cervical dystonia, hyperhidrosis as well as synkinesis following facial surgery.[6,7]

A previous research reported bone loss in alveolar and condylar areas of the rat mandible just after 4 weeks of unilateral injection of type A botulinum toxin into both the masseter and temporalis muscles.[8,9] There is still insufficient knowledge about exact bone changes and how these undesired consequences can be eliminated.[3] After the use of botulinum toxins in the muscles of mastication, condylar bone loss can be an

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Received: 06 Jan 2021; revision received: 01 Jul 2021; accepted: 07 Jul 2021

undesired result. Inactivation of masseter muscle from botulinum toxin type A injection procedure in a rabbit study resulted in significant cortical and trabecular bone loss in the mandibular condyle.[10] The subchondral bone of the mandibular condyle has been found to have reduced cell differentiation and accelerated apoptosis resulting in cell death.[8]

The purpose of this study was to evaluate the effects of muscle atrophy on the underlying bone secondary to intramuscular injection Botox. This study highlighted the effects of chemo-denervation with botulinum toxin type A that result in mandibular bone loss. Considering that, for its development and homeostasis, the operation of the masticatory muscles is required by the temporomandibular joint and the effect of the Botulinum toxin type A intervention on the masticatory apparatus is significantly linked to this phenomenon. The general population need to make informed choices regarding minimally invasive cosmetic procedures such as injection Botox that may lead to loss of muscle, subsequent bone loss and joint damage.

METHODOLOGY

A laboratory based experimental study, in collaboration with the National Institute of Health (NIH), Islamabad, was conducted in Department of Anatomy, Army Medical College, NUMS, Rawalpindi, from June to August 2020, following approval from the Ethics Review Committee of Army Medical College (ERC/ID /103), Rawalpindi, and the National University of Medical Sciences Islamabad. The non-probability consecutive sampling technique was used.

Inclusion Criteria: Adult female, non-pregnant sprague dawley rats of 10 weeks age, weighing 200-250gm were included in the study.

Exclusion Criteria: Mice with any obvious injury and disease were excluded.

Thirty rats were housed in separate individually marked cages in standardized environment of animal house of NIH, Islamabad. The air in the room was filtered at the temperature of 22°C ± 2°C and 50% ± 10% relative humidity with the 12-hours dark-light sleep cycle throughout the duration of experiment. Rats were divided into three groups. Each group had 10 rats. Rats were fed with laboratory-standardized diet provided by NIH and water was provided ad libitum. Animals were acclimatized for 1 week prior to the beginning of the study. In the first and third week, 2.5U of Botulinum toxin injection was administered on the left side of the masseter muscle in groups C under general anesthesia with Isoflurane (Restane Solution 100ml). In the sham group, same dosage of normal saline was administered on the left side of the masseter muscle.[11]

Using Triple beam balance, initial and final animal weights were recorded. By measuring the difference between final weight and initial weight, total body weight shift was derived.

Weight of the left mandible was recorded using a digital precision balance. The following bone measurements were taken by Vernier calipers and scale: 1) Mandibular length (Condylion, the craniometric point of the tip of the mandibular condyle, to incisor process), 2) Condyle head length (Distance from the condylion perpendicular to a line traced from the sigmoid notch to the maximum depth in the concavity of ramus of the mandible), 3) Condyle head width (The distance of the condylar articular surface from the anterior most point to the posterior most point). Measurements were made and the mean for each specimen was determined.[2]

Data was analysed by using Statistical Package for Social Sciences (SPSS) version 23. Quantitative parameters were expressed as Mean ± SD, i.e., body weight change and morphometric measurements of the mandible. Analysis of variance (ANOVA) was used to determine the significant difference and the *p*-value ≤0.05 was considered as significant.

RESULTS

During our study period, 10 out of 30 rats with average weight of 215 ± 3 gms were injected with 2.75 U of injection botox in left masseter muscle in first and 3rd week of the experiment while the 10 rats of group-B were sham injected with the equal volume of normal saline.

Mean weight gain in control group-A and sham group-B was 11.90 ± 2.132gm and 11.40 ± 1.776gm respectively. In experimental group-C mean weight gain was 2.20 ± 1.033gm. Comparison between groups for weight change was done which showed significant results (*p*-value <0.01). Inter-group comparison between control group-A and experimental group-C showed highly significant results (*p*-value <0.01) as well as between sham group-B and experimental group-C (*p*-value <0.01) (Table).

The mean weight of the left mandible in control group-A and sham group B was 0.846 ± 0.042 gm and 0.837 ± 0.051 gm respectively. In experimental group-C the mean weight of the left mandible was 0.804 ± 0.051

gm. Difference between groups was analyzed which showed insignificant results (Table).

Table: Comparison between control Group-A, Sham Group-B and experimental Group-C.

Parameters	Groups Distribution (Mean ± SD)			*p*-value
	Group-A, n=10	Group-B n=10	Group C, n=10	
Weight change (gm)	11.90 ± 2.132	11.40 ± 1.776	2.20 ± 1.033	<0.01
Weight of the left mandible (gm)	0.846 ± 0.042	0.837 ± 0.051	0.804 ± 0.051	0.146
Mandibular length (mm)	30.41 ± 0.562	30.59 ± 0.514	30.17 ± 0.439	0.192
Condylar head length (mm)	8.69 ± 0.059	8.64 ± 0.076	8.67 ± 0.0585	0.250
Condylar head width (mm)	5.59 ± 0.415	5.55 ± 0.419	3.71 ± 0.234	<0.01

Inter-Group Comparison (Post Hoc Analysis)

Parameters	Group-C vs Group-A	Group-C vs Group-B	Group-B vs Group-A
Weight change	<0.01	<0.01	0.792
Condylar head width	<0.01	<0.01	0.961

The mean mandibular length for control group-A, sham group-B, experimental group-C were 30.41 ± 0.562 mm, 30.59 ± 0.514 mm and 30.17 ± 0.439 mm respectively and the results were statistically insignificant (*p*-value=0.114).

The condylar head length for control group-A and sham group-B had the mean value of 8.53 ± 0.258 mm and 8.58 ± 0.185mm respectively. The mean condylar head length in experimental group-C was 8.40 ± 0.235 mm. The difference between the groups was statistically significant (*p*-value=0.036).

The control group-A and sham group-B had the mean condylar head width of 5.59 ± 0.415 mm and 5.55 ± 0.419 mm respectively. The mean condylar head width in experimental group C was 4.09 ± 0.174 mm. Difference between groups was highly significant (*p*-value <0.001).

DISCUSSION

Out of all the minimally invasive procedures available in the market today, botox is the most widely used product for cosmetic purposes.[11] The use of botox appears to be the most common minimally invasive procedure conducted by plastic surgeons and has developed into a billion dollar industry over the past decade.[12] Durand *et al*, supported the idea that repeated type A botulinum toxin injection is likely to be responsible for both therapeutic and unintentional atrophy of the muscle with 30% masseter muscle reduction after 3 months of injection and up to 13.4% reduction in muscle volume even after restoring the muscle function using electromyographic data.[13] Chemical denervation induced by botox injection paralyses the muscle and induces inflammation, satellite cell activation, oxidative stress, atrophy and metal ion imbalance in a variety of muscle events.[14] The diminished muscle function due to disuse of muscle precipitates reduced bone mass through aggressive bone resorption in contrast to altered osteoblast function in mice after 21 days of injection despite the weight bearing function was restored within 14 days. The mass of the muscle was diminished by -47.3% and -59.7% for quadriceps and calf muscles respectively vs. saline mice. The ratio of bone volume to tissue volume (BV/TV) within the distal femoral epiphysis and proximal tibial metaphysis of Botox injected limbs was also observed to be reduced by 43.2% and 54.3%, respectively.[15] Paralysis, fatigue, and atrophy are the symptoms of botox on skeletal muscles, although there are side effects that could impair bone homeostasis. A fine regulation between muscles and bones is preserved by the musculoskeletal system.[16] The bone loss induced by botulinum toxin type A did not differ from that induced by aging process in adults, progressing from osteopenia to osteoporosis.[17]

Aim of the current study was to assess the side effects of disuse of masseter muscle induced by injection botox on the mandible. All the animals remained healthy throughout the experiment and the survival rate was 100%. Group-B was sham injected by equal volume of normal saline in left masseter muscle. Intergroup comparison of control group A and experimental group B yielded insignificant results. This observation was reinforced by a previous study of Kim *et al* which yielded insignificant results for all the parameters (trabecular thickness, bone volume and condylar volume) when control group was compared with sham injected masseter muscle on the opposite side of the experimental side.[11]

At the beginning of the experiment, the rats were 10 weeks old. They were still in their growing age. The weight at the start of the experiment and before the sacrifice was recorded. The results of weight gain in group control group-A and sham group-B when compared with experimental group-C, were highly significant (*p*-value <0.001) which were not consistent with the previous study according to which there was no significant weight change between the experimental

group and control group after botox injection in masseter muscle.[18]

The morphometric measurements for the mandible gave no significant results when mandibular length and condylar head length were compared between control group-A, sham group-B and experimental group C. The results of condylar head width in experimental group C when compared with control group-A and sham group-B revealed statistically highly significant results (p-value <0.001) and coincided with the previous study that demonstrated decreased width of the condylar head at the injected side.[2]

ACKNOWLEDGMENT

The authors were highly grateful to the National University of Medical Sciences, Islamabad for providing financial support for our project

CONCLUSION

Injection botox significantly decreases the morphometric measurements of the mandible. It also causes a decrease in total body weight gain of animals due to inadequate intake secondary to muscle of mastication in growing rats.

Conflict of Interest: None.

Authors' Contribution

MI: Principal Auther, MRBK: Substantial contribution to the conception, design & analysis, KQ: Substantial contribution to the conception, SS: Analysis and interpretation of data, MFA: Analysis and interpretation of data, IZ: Analysis and interpretation of data.

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