A Pilot Study Comparing the Clinical Efficacy of Freshly Reconstituted and Botulinum Toxin A Reconstituted 1, 2 And 3 Months before Application in the Treatment of Axillary Hyperhidrosis*

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ABSTRACT

Background: Most manufacturers of commercially available botulinum toxin A (BTX-A) recommend that the vials should be used within 24 hours after reconstitution to ensure efficacy, which in some instances would mean wastage of remaining reconstituted solution. Several studies have evaluated the efficacy of stored reconstituted BTX-A and have concluded that the use of BTX-A reconstituted and refrigerated for up to 6 weeks prior to administration does not significantly alter its efficacy in the treatment of facial rhytides.

Objectives: Our study aimed to compare the clinical efficacy and safety of freshly reconstituted BTX-A and BTX-A reconstituted 1, 2 or 3 months prior to administration in the treatment of axillary hyperhidrosis.

Methodology: Patients with primary axillary hyperhidrosis were enrolled in this pilot study. Freshly reconstituted BTX-A and BTX-A reconstituted 1, 2 and 3 months prior were administered in 4 pre-determined areas in the same patient. The degree of hyperhidrosis was assessed subjectively using Hyperhidrosis Disease Severity Scale (HDSS) and objectively using Minor's iodine starch test followed by Sweating Intensity Visual Scale (SIVS) at 0, 2, 6 and 12 weeks after administration.

Results: Five patients were enrolled in the study. Kruskall-Wallis test showed that HDSS at baseline was significantly different from follow-up periods with noted improvement from baseline to 2 weeks follow-up. Using Kruskall-Wallis test, SIVS was found to be not significantly different among these 4 treatment areas. In addition, significantly improved SIVS scores were noted as early as 2 weeks after administration in all 4 areas of treatments. There were no noted adverse effects in all patients at baseline and at all follow-up visits.

Conclusion: The clinical efficacy and safety of BTX-A reconstituted 1, 2 and 3 months prior to administration is comparable to that of freshly reconstituted BTX-A in the treatment of axillary hyperhidrosis.

Keywords: stored botulinum toxin A, reconstituted botulinum toxin A, axillary hyperhidrosis

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INTRODUCTION

Botulinum toxin A (BTX-A) is commonly used in dermatology for facial rejuvenation and in the treatment of axillary and palmoplantar hyperhidrosis. The use of freshly reconstituted BTX-A in the treatment of axillary hyperhidrosis has been established by several studies and is associated with reduced disease severity and improvement of quality of life.[1-7]

One of the drawbacks with the use of BTX-A is its cost. Adding to this is the fact that manufacturers recommend that once reconstituted, the vial should be consumed within 24 hours and any remaining solution be discarded.[8-10] Because of this, a common practice among dermatologists is to pool patients before reconstituting a vial of BTX-A, otherwise, even if the entire vial is not consumed in 1 treatment session, one patient will have to pay for the cost of an entire vial.

Because the recommendation that reconstituted vials should be discarded after 24 hours would mean wasting an expensive product in most instances, there is great interest in the methods of storing reconstituted BTX-A. Several studies have investigated the efficacy of reconstituted BTX-A stored at 4˚C for up to 6 weeks and have concluded that it is equal to that of freshly reconstituted BTX-A, at least in the treatment of facial rhytides.[2,11-13]

In our literature search, there is no published study investigating the clinical efficacy and safety of reconstituted BTX-A stored at 4˚C for more than 6 weeks before use in the treatment of axillary hyperhidrosis. This study aims to compare the clinical efficacy and safety of freshly reconstituted BTX-A and BTX-A reconstituted 1, 2 and 3 months before application in the treatment of axillary hyperhidrosis, which in the future, may contribute in lowering treatment costs with BTX-A.

METHODOLOGY

Study design and study population

A non-blinded, non-randomized, controlled pilot study was conducted at the Jose R. Reyes Memorial Medical Center Department of Dermatology from April 2016 to December 2016 to compare the clinical efficacy of BTX-A reconstituted at 0, 1, 2 and 3 months before application in the treatment of axillary hyperhidrosis. This study was conducted with the approval of the Institutional Review Board and in compliance with Good Clinical Practice guidelines. This study was partially funded by a research grant from Philippine Academy of Dermatologic Surgery Foundation Inc. (PADSFI).

Patients aged 18 to 40 years old, male and female, who were diagnosed with primary axillary hyperhidrosis (see Appendix A) and with moderate to severe hyperhidrosis, defined as having a score of 3 or 4 using the Hyperhidrosis Disease Severity Score (HDSS) (see Table 1), were included in the study.

Patients with concurrent treatment for hyperhidrosis, including aluminum chloride 40%, iontophoresis, anticholinergic drugs and BTX-A within the last 12 months; infection or dermatosis over the axillae; pregnancy or lactation; and negative Minor's iodine starch test were excluded. Patients with regular use of commercially available anti-perspirant were instructed to discontinue its use 3 days before the administration of BTX-A and throughout the duration of the study.

Materials

One 100-unit vial of BTX-A was reconstituted by the co-investigator (AET) by adding 2.5 mL of bacteriostatic 0.9% sodium chloride with resulting dose unit of 4 units/0.1 mL. In addition to freshly reconstituted BTX-A that is reconstituted on the day of administration, vials of BTX-A were reconstituted and refrigerated at 4˚C for 1, 2 and 3 months before the scheduled date of BTX-A administration. These vials were labeled 0, 1, 2 and 3 corresponding to the months the reconstituted vials were stored.

Study intervention and assessment

After screening of eligible participants, the primary investigator (CMP) explained the study to the participants and obtained informed consent. Detailed history was taken and the patients were asked to answer the HDSS (see Table 1). Patients were then instructed to discontinue use of any commercially available anti-perspirant prior to the procedure and were scheduled to follow-up on a specified date.

Minor's iodine test was performed by CMP to determine the location of hyperactive sweat glands before administration of BTX-A and to document improvement in hyperhidrosis on follow-up visits. Minor's iodine test was performed by painting the axillae with 10% iodine solution and applying cornstarch powder after. Subjects were then exposed to room temperature of 30-35˚C and observed after 30 minutes. Sweating was indicated by the onset of a dark-blue color.[5]

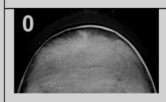
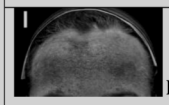
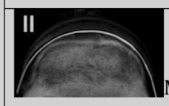
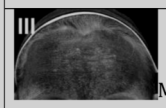
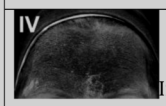
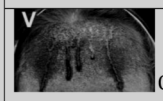
Administration of BTX-A was performed by CMP under the supervision of AET. Two areas per axilla, at least 2 cm x 2 cm, with the highest intensity of sweating as determined using Minor's iodine test, were marked and assigned to receive either freshly reconstituted BTX-A (labelled 0), or BTX-A reconstituted 1 month, 2 months or 3 months before application (labelled 1, 2 or 3, respectively). Photos of the labelled treatment areas subjected to Minor's iodine test were taken at baseline and at all follow-up periods. Injection points, 1 cm. apart, were marked using a fine-tipped marking pen in each treatment area. The treatment areas were then wiped using gauze with sterile water to remove the iodine and cornstarch. Two units (0.05 mL) of BTX-A were administered intradermally in each injection point using tuberculin syringe with gauge 30 needle. No anesthesia was used.

Patients were asked to follow-up 2 weeks, 6 weeks and 12 weeks after administration of BTX-A to assess the reduction of hyperhidrosis. The degree of hyperhidrosis of all the treatment areas were evaluated by 3 independent investigators (senior residents) using SIVS (see Table 2) at baseline and on follow-up visits through photos. Patients were also asked to answer the HDSS on all follow-up visits. Adverse effects, which included pain after injections, bruising and infection, were also noted.

Table 1. Hyperhidrosis Disease Severity Scale (HDSS)[5]

Condition	Score
My axillary sweating is never noticeable and never interferes with my daily activities	1
My axillary sweating is tolerable but sometimes interferes with my daily activities	2
My axillary sweating is barely tolerable and frequently interferes with my daily activities	3
My axillary sweating is intolerable and always interferes with my daily activities	4

Table 2. Sweating Intensity Visual Scale (SIVS)[14]

	Grade
 Minimal or no sweating	0
 Initial, discrete sweating	I
 Mild sweating	II
 Moderate sweating	III
 Intense sweating	IV
 Over-sweating	V

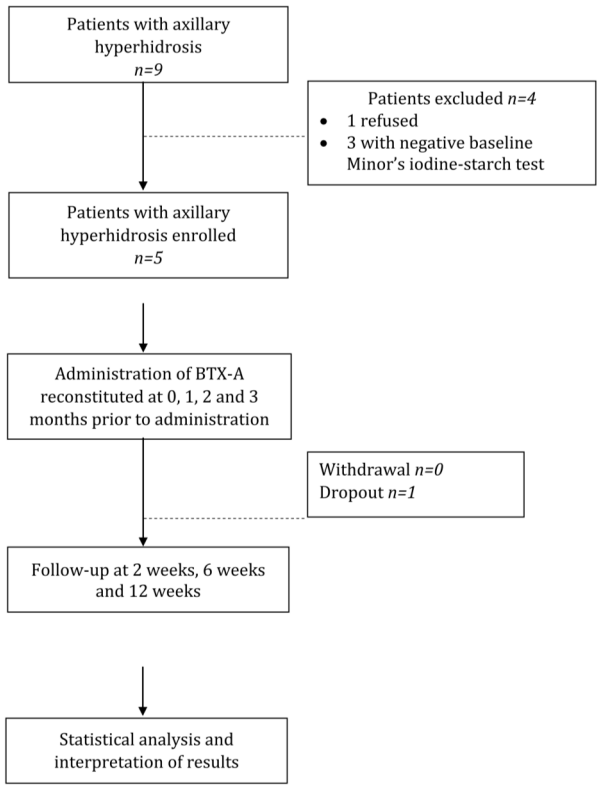
Statistical analysis

Descriptive analysis of the data was performed by determining the percentage or mean with standard deviation to describe the demographic profile of all the subjects, which include age, gender and duration of hyperhidrosis. Means of SIVS scores with standard deviation were also calculated during each follow-up visit.

For inferential statistics, Kruskall-Wallis test at 5% level of significance was performed to determine if there is significant difference in the mean SIVS scores for areas treated with BTX-A reconstituted 0, 1, 2 and 3 months prior to administration. This test was also used to determine if there is significant improvement in the mean HDSS scores during follow-up visits.

Proportion of those who experienced adverse effects was measured to determine the percentage of subjects who experienced the adverse effects.

Figure 1. Schematic diagram of the methodology

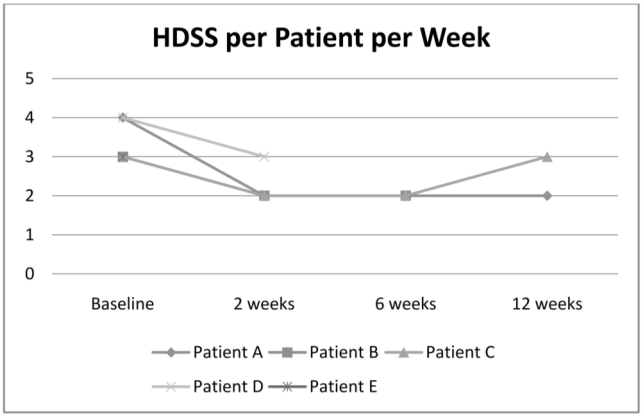


RESULTS

A total of 5 patients, all female, diagnosed with primary axillary hyperhidrosis at Jose R. Reyes Memorial Medical Center Department of Dermatology, were included in the study. The ages ranged from 20 to 29 years old, with mean age of 22.6 years old (SD of 3.78). Duration of hyperhidrosis of these patients ranged from 2 to 10 years, with mean duration of 6.8 years (SD of 3.96).

The severity of hyperhidrosis of these patients was measured using the HDSS where 1 is least severe and 4 is most severe. Patients A and E both have HDSS of 4 at baseline while patients B, C and D have HDSS of 3 at baseline. However, patient E was lost to follow up at 2 weeks and all succeeding follow-ups. HDSS of patient A decreased by 2 scales from baseline, while HDSS of the other 3 patients decreased by 1. HDSS of patient C returned to baseline at 12 weeks follow-up. (see Figure 2).

Figure 2. HDSS of patients at baseline and at 2, 6 and 12 weeks follow-up



To determine if mean HDSS significantly improved from baseline, Kruskall-Wallis test at 5% level of significance was used. Mean HDSS was 3.4 at baseline, 2.2 after 2 weeks, 2 after 6 weeks, and 2.5 after 12 weeks. Results of the test showed that HDSS at baseline was significantly different from follow-up periods (p-value=0.031) with noted improvement from baseline to 2 weeks follow-up (see Table 3).

Table 3. Kruskall-Wallis test - mean HDSS at baseline and after 2, 6 and 12 weeks of administration of BTX-A

Variable	#	Follow-Up				P-Value
		Baseline	2 weeks	6 weeks	12 weeks	
HDSS	5	3.4 ± 0.55	2.2 ± 0.50	2.0 ± 0.00	2.5 ± 0.71	**0.031***

ns – not significant * - significant at 5%

Three non-blinded independent investigators assessed the sweating intensity after Minor's iodine starch test at baseline and during follow-up visits using the SIVS, which ranged from 0 (minimal sweating) to 5 (over-sweating). The average SIVS scores were calculated and recorded.

At baseline, the areas that received freshly reconstituted BTX-A have mean SIVS of 2.7; while mean SIVS were 3.3, 2.8, and 3.3 for those areas that received BTX-A reconstituted 1 month, 2 months, and 3 months prior to administration, respectively. Mean SIVS scores were 0, 0.25, 0.25 and 0.12 at 2 weeks; and 0.33, 0, 0.83 and 0 at 6 weeks; and 0, 0, 0.50 and 0 at 12 weeks for BTX-A reconstituted 0, 1, 2 and 3 months prior to administration, respectively.

Using Kruskall-Wallis test at 5% level of significance, SIVS was found to be not significantly different among these 4 treatment areas at baseline (p-value=0.792), at 2 weeks follow-up (p-value= 0.758), at 6 weeks follow-up (p-value=0.224) and at 12 weeks follow-up (p-value=0.392). This means that the efficacy of BTX-A reconstituted 1, 2 and 3 months prior to administration is comparable to freshly reconstituted BTX-A. In addition, significantly improved SIVS scores were noted as early as 2 weeks after in all 4 treatment groups (see Table 4).

Table 4. Kruskall-Wallis test - mean SIVS for BTX-A reconstituted at 0, 1, 2 and 3 months prior to administration at baseline and at 2, 6 and 12 weeks follow-up

Follow-Up	#	Mean ± SD, SIVS				P-Value
		BTX-A (0)[a]	BTX-A (1)[b]	BTX-A (2)[c]	BTX-A (3)[d]	
Baseline	5	2.70 ± 1.30	3.30 ± 0.45	2.80 ± 1.40	3.30 ± 0.45	**0.792[ns]**
2 weeks	4	0.00 ± 0.00	0.25 ± 0.50	0.25 ± 0.50	0.12 ± 0.25	**0.758[ns]**
6 weeks	3	0.33 ± 0.58	0.00 ± 0.00	0.83 ± 1.04	0.00 ± 0.00	**0.224[ns]**
12 weeks	1	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.71	0.00 ± 0.00	**0.392[ns]**
P-Value		**0.012***	**0.010***	**0.036***	**0.013***	

a - Freshly reconstituted BTX-A

b - Reconstituted BTX-A stored for 1 month

c - Reconstituted BTX-A stored for 2 months

d - Reconstituted BTX-A stored for 3 months

ns – not significant * - significant at 5%

In all patients, there were no noted pain after injections, bruising and infection at baseline and at all follow-up visits.

DISCUSSION

Most manufacturers of BTX-A recommend using the toxin within 24 hours of reconstitution to ensure maximum efficacy and that any remaining solution should be discarded.[8-10] Because 1 100-unit vial is not always consumed in most circumstances (e.g., rhytides in the upper part of the face require approximately 70 units), the storage of reconstituted BTX-A has been a subject of great interest, with several studies evaluating the clinical efficacy of stored BTX-A.[2,11-13]

In our knowledge, this is the first study investigating the efficacy of BTX-A reconstituted and stored at 4˚C for more than 6 weeks. In addition, this is the first study investigating the efficacy of stored reconstituted BTX-A in the treatment of axillary of hyperhidrosis, as previous studies were conducted in patients with facial rhytides. In the authors' opinion, treatment of hyperhidrosis provides a more superior assessment of the efficacy of BTX-A, compared to rhytides, since Minor's iodine starch test and SIVS, with its 5-point visual scale, can be used in hyperhidrosis to objectively assess the decrease in sweating. The authors opted to treat only axillary hyperhidrosis and not palmoplantar hyperhidrosis for this pilot study because the latter is associated with more pain on injection and higher incidence of side effects, including weakness.

The results of our study showed that BTX-A reconstituted and stored at 4˚C for 1 month, 2 months and 3 months have comparable efficacy to freshly reconstituted BTX-A in the treatment of axillary hyperhidrosis at 2 weeks, 6 weeks and at 12 weeks after administration. In addition, there were no noted side effects in all patients at baseline and at all follow-up visits.

Because this pilot study showed that reconstituted BTX-A can be stored for at most 3 months before administration, the authors recommend that a randomized controlled trial be conducted to compare the clinical efficacy and safety of freshly reconstituted BTX-A and BTX-A reconstituted 3 months prior to administration to strengthen our findings.

In conclusion, the clinical efficacy and safety of BTX-A reconstituted 1, 2 and 3 months prior to administration is comparable to that of freshly reconstituted BTX-A in the treatment of axillary hyperhidrosis.

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APPENDICES

Appendix 1. Minor's iodine test of patient A at baseline and at 2 weeks, 6 weeks and 12 weeks follow-up. Treatment areas were labelled 0 for area receiving freshly reconstituted BTX-A, 1 for reconstituted BTX-A stored for 1 month, 2 for reconstituted BTX-A stored for 2 months and 3 for reconstituted BTX-A stored for 3 months